# THE SECOND WAVE OF EARTHWORM INVASION: INTERSPECIFIC INTERACTIONS, SOIL MICROBIAL COMMUNITIES, AND CARBON CYCLING

by
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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
September, 2015

# ABSTRACT

In temperate soils, earthworms are significant ecosystem engineers. Recent studies of North American earthworms focusing on invasive European species have demonstrated that invasive European earthworms redistribute nutrients in different pools in the soil and accelerate flux rates among the pools, leading to changes in ecosystem functions. In recent years, a group of Asian invasive earthworms, *Amynthas*, has been widely reported to be invading forests already inhabited by European species in the Mid-Atlantic region, causing a "second wave of invasion" where the soil ecosystem, already modified by European species, is going through another transition. The objective of this thesis is to understand how the invading Asian species affect the European and native earthworms through interspecific interaction and how these interactions alter soil microbial communities and C dynamics. A revised checklist of species and a new key and diagnosis to species in the genera *Amynthas*, *Metaphire*, *Pithemera* and *Polypheretima* recorded in North America are presented based on published records and inspecting specimens archived at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) from earthworm tissues indicated that *Amynthas hilgendorfi*, one of the most common Asian invaders, competes with European species for food resources, providing the first direct evidence of interspecific competition in earthworms. Compared to European and native species, *A. hilgendorfi* has stronger effects on soil microbial communities and C dynamics, leading to reduced bacteria-to-fungi ratio, increased fresh soil organic matter translocation into subsurface soil, and reduced litter C-derived soil respiration. Altogether, through outcompeting

European species, modifying soil microbial community structures and altering C dynamics, *A. hilgendorfi* invasion may shift the ecosystem into an alternative steady state.

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# ACKNOWLEDGEMENTS

For a graduate student, it is always a blessing to have an advisor who is supportive and thinks for the student. I am really lucky to have one in Katalin Szlavecz (Kathy). Kathy is extremely willing to devote resources, which ecologists usually do not have a lot, for my intellectual development. Naomi Levin and Linda Hinnov are my committee members and Naomi is also the second reader of my thesis. They are extremely good at getting me excited about my own research, and I was always fully geared up after each semi-annual review at the end of each semester.

Jeffrey Buyer and Timothy Filley are my collaborators and co-authors of one of my paper. Their comments were crucial during the process of data analysis and manuscript preparation. Members of Kathy's lab, Michael Bernard (Mike), Scott Pitz, Natalie Bray, Jacob Rode, Adam Dec, Lijun Xia, Jerry Burgess and Xu Yang, provided me various assistances and/or suggestions. Scott and Mike are also co-authors in one of my paper and their contributions are a crucial part of my study as a whole. I also received a lot of help from undergraduate students, especially Ziwei He, Zachary Ferguson and Kelly Baker.

The staffs in the Department of Earth and Planetary Sciences, including Kristen Heisey, Kimberly Trent, Jean Light and Teresa Healy, worked behind the scene. A lot of things were not possible without them. Our department is small but extremely supportive and welcoming. EPS Summer Field Funds is the most important funding source of my thesis

and is what made my work possible. Funding to this work was also provided by National Science Foundation (EEC-0540832).

Chuan-Chin Huang discussed with me for hours after hours on the phone on data analysis. Mac Callaham, Bruce Snyder, Sam James, Rob Blakemore, Csaba Csuzdi provided a lot of insights on the ecology, taxonomy and systematics of earthworms. Jay O'Neill, Pavlo Bohutskyi and Alan Stone helped with using freeze drier. Christine France helped with stable isotope measurement. Stanley Tesch helped with PLFA analysis. Melissa McCormick and Dennis Whigham provided suggestions about fieldwork at SERC. Jim Grace helped with structural equation modeling. Insights from Heikki Setala on soil ecology, nematodes and career decision are both crucial and very valuable. Huei-Ping Shen kept me refreshed on earthworm taxonomy.

Friends from both inside and outside the department are the most important reason why my life in Baltimore is awesome. Yes, I do mean awesome, literally. I decided not to name anyone here because the list goes on and on and does not seem to be ending. Whoever believes his or her name should be included here, I assure you that your name is definitely in my mind.

The unconditional support from my parents, my sister and my wife is what kept me afloat and going during my six years at Hopkins. My wife Jia-Hsing Wu visited me in Baltimore at least once a year and helped me with my experiments whenever I need help. Her love is the most precious gift I have ever received from God. I will always remember my sister's dog Mini, who left us and went back to the Father two days after my thesis presentation.

Last but not least, thank you God. Your voice is what I have been trying to follow. My mother once told me, "Listen to God and distinguish what he sends you." Thanks for giving me signs when I need them the most. Please keep guiding me throughout my life.

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# 1. INTRODUCTION

Invasive organisms affect ecosystem functioning by altering nutrient pool size and flux rates through translocation and transformation, and/or changing the input and output at the ecosystem level (Jones et al. 1997; Ehrenfeld 2010). Three major mechanisms have been identified for these impacts: 1) Individual species can have strong effects; these species are often recognized as ecosystem engineers, keystone species, foundation species, etc.; 2) Species in the same taxonomic or functional group may have distinct biological traits that affect processes related to ecosystem functions differently; 3) Some species affect ecosystem functions by altering the structure of food webs. Many studies have focused on the concepts of ecosystem engineers and keystone species, as well as functional traits of invasive plants. However, studies focusing on species-specific effects of animal invaders are rare, and mechanisms through which invasive terrestrial invertebrates alter ecosystem functions are poorly understood, especially in belowground ecosystems. It is also often unclear whether the effect of the invasive species is a result of their higher densities and biomass, or individuals of invasive species have traits that cause the observed ecosystem impacts, or some combination of the two (Ehrenfeld 2010).

In temperate soils, earthworms are the most significant ecosystem engineers, organisms whose behavior changes the physical characters of their environment. Recent literature in this field has focused on non-native lumbricid earthworms of European origin and their effects on temperate forests in North America (Bohlen et al. 2004a; Frelich et al. 2006; Hendrix et al. 2008, Szlavecz et al. 2011). By consuming leaf litter and soil organic matter, invasive earthworms redistribute nutrients in different pools in

the soil and accelerate flux rates among the pools, leading to reduction of the understory vegetation and the leaf litter layer (O horizon), and at the same time increasing the thickness of organic soil (A horizon). These changes in nutrient pools and modification of habitats have tremendous effects on the soil food web, causing changes in abundance and composition in the mesofauna communities, and are hypothesized to result in structural shifts from fungal pathway-dominated systems to bacteria pathway-dominated systems (Bohlen et al. 2004b).

Another group of invasive earthworms, *Amynthas*, remained largely unnoticed by most North American ecologists until about a decade ago (Burtelow et al. 1998; Callaham et al. 2003), even though their presence in North America has been documented repeatedly since 1867 (Gates 1937, 1958, 1982). Today their broad distribution and often visible and dramatic effect on the forest floor has generated interest in *Amynthas* ecology among both scientists and land managers. European lumbricids have long been established in the Mid-Atlantic region of the US and at certain sites coexist with native earthworms. Multiple species of *Amynthas* have caused a "second wave of invasion" where the soil ecosystem, already modified by European lumbricids, is going through another transition. However, with little ecological knowledge on species-specific earthworm life history and biology about the Asian *Amynthas* (e.g. Garcia and Fragoso 2002; Snyder et al. 2009, 2011; Zhang et al. 2010), even in its native continent (Kawaguchi et al. 2011), it is difficult to predict how this second wave of invasion will change ecosystem functions in the Mid-Atlantic.

## THE NATIVE EARTHWORM FAUNA AND EUROPEAN LUMBRICIDAE INVASION IN THE EASTERN DECIDUOUS FORESTS

The native earthworm fauna of the Eastern deciduous forests of the United States is composed of about 20 nominal species in the genera *Bimastos* and *Eisenoides* of the family Lumbricidae, in the genus *Diplocardia* of the family Acanthodrilidae, and in the genus *Komarekiona* of the family Komarekionidae (Reynolds 2010, 2011). Although our knowledge about these species is limited, it is safe to assume that their abundances are usually low, regardless of the presence or absence of non-native species at the same localities. The native earthworm communities in this region are also characterized by small to medium-sized epigeic and endogeic species, lacking both large-bodied and anecic species. For instance, at the Hawk Mountain Sanctuary, Kempton, PA, only three earthworm species are found within the non-edge part of the forests, *Eisenoides carolinensis*, *E. loennbergi*, and *Bimastos palustris*, all of which are native. The total earthworm densities are generally below 10 individuals/$m^2$, with the highest density recorded being 28 and 16 individuals/$m^2$ for *E. carolinensis* and *B. palustris*, respectively (Szlavecz et al., unpublished data). In most samples, only *E. carolinensis*, an epigeic or epi-endogeic species, was found. Similarly, at the Mountain Lake Biological Station, located in the Virginia portion of the southern Appalachian Mountains, only the native species *E. carolinensis* (epigeic or epi-endogeic) and *Diplocardia* spp. (endogeic) were found, with an average density of only 7.5 individuals/$m^2$ (Rearick et al. 2011).

Sixteen invasive European lumbricid species have been reported in North America; these species are *Allolobophora chlorotica*, *Aporrectodea longa*, *Ap. rosea*, *Ap.*

*trapezoides*, *Ap. tuberculata*, *Ap. caliginosa*, *Dendrobaena octaedra*, *Dendrodrilus rubidus*, *Eisenia fetida*, *Eiseniella tetraedra*, *Lumbricus castaneus*, *L. friendi*, *L. rubellus*, *L. terrestris*, *Octolasion cyaneum*, and *O. lacteum* (Reynolds and Wetzel 2008). Some of these species have special habitat requirements and are not found in forests, grasslands, and agriculture fields, but most of them survive and reproduce in one or all of the above habitats. Many more non-native European species have been reported but are not considered invasive, as there are no confirmed reports about established populations and range expansion. In Eastern deciduous forests, the invasive earthworm communities are usually composed of species of all the three functional groups. For instance, the earthworm assemblage in one of the forest patches at the Smithsonian Environmental Research Center, MD has the epi-endogeic species *L. rubellus*, the endogeic species *Ap. caliginosa*, *Ap. rosea*, *O. cyaneum*, and *O. lacteum*, and the anecic species *L. friendi* (Szlavecz et al. unpublished data). Abundances of earthworms vary a lot, but densities more than 100 individuals/$m^2$, an order of magnitude higher than native earthworm communities (see above), are common (Eisenhauer et al. 2007; Szlavecz and Csuzdi 2007). The high density and biomass of non-native earthworm communities may be related to biological traits like high cocoon production rates and/or parthenogenesis, and may be responsible for the transformation of forest floor and soil characteristics.

In North American temperate deciduous forests invasive earthworms have been reported to lead to major alterations in soil physical and chemical properties, such as increased bulk density (Hale et al. 2005), reduced water retention capacity (Dobson and Blossey 2015) and changing pH (Eisenhauer et al. 2007), and have dramatically affected several aspects of ecosystem functioning. Among the most noticeable impacts are the

transformation of forest floor through accelerating leaf litter disappearance and eliminating understory vegetation, and the redistribution of soil organic matter through foraging, casting, and burrowing. Field studies have consistently reported that these activities lead to a reduced nutrient pool in the soil at sites heavily invaded by earthworms (Bohlen et al. 2004a, b; Eisenhauer et al. 2007; Crumsey et al. 2013), but the results on other nutrient cycling parameters have been inconclusive. Szlavecz et al. (2011) reported higher soil respiration in plots with higher earthworm abundance in temperate deciduous forests at the Smithsonian Environmental Research Center, Maryland, suggesting increased C efflux rate due to earthworm invasion. Alban and Berry (1994) reported C loss of about 600 kg per hectare per year between 1979 and 1992, spanning a period of earthworm population size increase. Similar increases were also reported in rates of N mineralization (Steinberg et al. 1997; Willems et al. 1996). On the other hand, some studies reported neutral or negative effects on flux rate-related parameters. For instance, Bohlen and his colleagues reported no differences on soil respiration, N-mineralization, and nitrification in earthworm-invaded plots in New York (Fisk et al. 2004; Groffman et al. 2004). A recent meta-analysis concluded that the positive effects of invasive earthworms on soil respiration is transient and relative short-term, and may not represent the long-term effects (Lubbers et al. 2013).

In addition to quickly changing nutrient availability and flux rate over short time intervals, the long-term effects of earthworms on C cycling may involve different processes. Through their casting behavior, earthworms may increase soil organic matter stabilization by facilitating large macroaggregate (>2000 μm) formation and by incorporating soil organic matter into the aggregates. When compared to aggregates not

affected by earthworms, these large macroaggregates contain more stable microaggregates (53-250 μm) and more particulate organic matter within and between the microaggregates. The organic matter in these structures is well-protected from soil enzymes and micro-organisms, and thus more stable than unprotected soil organic matter (Bossuyt et al. 2004). Accordingly, the net effect of earthworms on soil organic matter dynamics is likely to be a combination of the long and short-term effects; it may be context-dependent; and it is still poorly understood.

The effects of earthworms on soil bacteria and fungi generally follow the translocation and transformation of resource due to earthworm activities. The drilosphere (casts, burrows, middens) are hotspots of microbial activity due to priming effects by earthworms, but the microbial activity drops quickly through time because of decreasing nutrient availability. The responses of microbial biomass in the soil column vary from site to site. Microbial biomass has been shown to decline in the forest floor due to elimination of the leaf litter layers (Dempsey et al. 2011). However, in the soil it may increase (Groffman et al. 2004) or decrease (Eisenhauer et al. 2007, 2011), leading to a net increase or decrease in total soil profile microbial biomass, respectively. The decrease of microbial biomass in the forest floor could in turn reduce the rates of microbially mediated leaf litter decomposition (Holdsworth et al. 2008). Earthworms are generally believed to have negative effects on the fungal communities due to physical disruption of fungal hyphae and elimination of the leaf litter layer, where a large fraction of the fungal community reside. The net result could be an increase in bacteria to fungi ratio in the bulk soil, a hypothesis supported by several laboratory studies (Scheu and Parkinson 1994; Butenschoen et al. 2007) and a recent field study (Dempsey et al. 2011).

## *AMYNTHAS* INVASION IN NORTH AMERICA

The *Amynthas* genus belongs to the family Megascolecidae. Composed of more than 400 nominal species, this genus is native to East and Southeast Asia, with about a dozen species considered cosmopolitan (Blakemore 2010a). In North America 10 species have been reported (Blakemore 2006; Reynolds and Wetzel 2008); these species are *A. agrestis*, *A. corticis*, *A. gracilis*, *A. hilgendorfi*, *A. hupeiensis*, *A. loveridgei*, *A. minimus*, *A. morrisi*, *A. rodericensis*, and *A. tokioensis*. Among them, *A. hilgendorfi*, *A. agrestis*, *A. gracilis*, *A. corticis* are the most common; the last two species listed are frequently and incorrectly cited under their junior synonyms, *A. diffringens* and *A. hawayanus*, respectively. The presence of these four species in forests and urban and suburban areas in the US has been widely reported in the last 20 years, frequently in high densities (Burtelow et al. 1998; Callaham et al. 2003; Snyder et al. 2011). However, their biology and ecology are poorly understood and their effects on the soil subsystem are essentially unknown.

According to the resource availability model (Davis et al. 2000), Eastern deciduous forests may have high potential community invasibility due to their high potential resource availability and increasing disturbances by human activity. Whether *Amynthas* will eventually invade a larger area in the Eastern deciduous forests and replace the non-native lumbricid earthworm fauna is still an open question. Invasive earthworms did not attract much attention until a decade ago; so the history and dynamics of the Aynthas invasion are not clear.

Several factors may be attributable to the successful invasion of *Amynthas* worldwide. Zhang et al. (2010) compared *L. rubellus* and *A. agrestis* and suggested that dietary flexibility may play an important role in the success of the latter. *A. corticis*, a species possibly originally from subtropical East Asia, has shown its ability to adapt to seasonal temperature fluctuations in different biomes. In tropical Northeast India, where most earthworms are active during the wet season, the population of *A. corticis* peaks during the drier winter months (Bhadauria and Pamakrishnan 1991). This life history trait may give the species the advantage of reducing interspecific competition with other earthworms and contribute to its global success. In temperate deciduous forests in the eastern US, *A. corticis*, like the common non-native Lumbricidae, is most active during summer and fall and survives the winter as adults (per. obs.). On the other hand, *A. hilgendorfi* and *A. agrestis*, both originally from Japan, survive the cold winter in the temperate area only as cocoons, a distinct life history trait that is absent among the common non-native lumbricids (Callaham et al. 2003; per. obs.).

Studies on *Amynthas* invasion in North America and in other regions of the world are scarce. *Amynthas agrestis* is an epigeic species originally from Japan. In addition to the Zhang et al. (2010) paper mentioned above, field data from the southern Appalachian Mountains in the US suggest that *A. agrestis* is an annual species, reaching its sexual maturity after late August and disappearing by the end of November (Callaham et al. 2003). Snyder et al. (2011) reported that increased density of this species is correlated with increased A-horizon aggregation and decreased thickness of leaf litter layer, and suggested that the invasion of this species may threaten native millipede diversity.

*Amynthas hilgendorfi*, originally from Japan, is another common invasive *Amynthas* in temperate regions. It is phylogenetically related to *A. agrestis* and is also morphologically and ecologically similar to the latter. Gut content and stable isotope analyses showed that this species is epigeic, with about 70% of its gut content being coarse organic material (Uchida et al. 2004). Its cast has higher pH, C and N content, and concentrations of dissolved organic carbon and $NH_4^+$ when compared to the surrounding soil. The fresh cast also has high rates of microbial respiration, nitrification, and N-mineralization, and more than 90% of the casts are water stable 24 hours after the casts are produced (Kawaguchi et al. 2011). Recently, Greiner et al. (2012) presented the first study focusing on invasion of this species in North America. Their results showed that when compared to *L. rubellus*, the presence of *A. hilgendorfi* leads to larger soil mineral N and P pools and increases soil aggregate size.

The limited number of studies that have been done on *A. agrestis* and *A. hilgendorfi* and on other invasive *Amynthas* species (Burtelow et al. 1998; Snyder et al. 2009) show that the effects of *Amynthas* on ecosystem function are similar to the effects of European lumbricids. However, this conclusion is likely premature and unfounded because it ignores the biological trait differences between these two groups. In addition, except the studies by Zhang et al. (2010) and Greiner et al. (2012), these two groups have never been compared under the same context (i.e. ecosystems, soil types, experimental setup, etc.), and we have almost zero knowledge about their interactions with the European lumbricids, which currently dominate the North American earthworm communities.

## RESEARCH OBJECTIVES

My research objective is **to understand how this second wave of invasion results in another transition of the soil ecosystem, and the mechanisms through which *Amynthas* leads to these changes.** My project addresses four main questions:

(1) How to distinguish and identify species in *Amynthas* and related genera occurring in North America?

(2) Under the ongoing invasion, do *Amynthas* compete with European species?

(3) How do different species, including those in the genus *Amynthas*, and their interspecific interactions affect soil microbial communities and C dynamics?

(4) In addition to functional group, is species identity important in understanding these processes?

One of the challenges of studying species-specific effects of *Amynthas* invasion is to identify the species involved in a certain context. This challenge is partially caused by the lack of a user-friendly guide and key to species in North America for the broader ecological research community. The available keys by Reynolds (1978) and Gates (1982) are out of date and focus too much on internal anatomy. This issue is addressed in **Chapter 2**.

The effects of a new invader on ecosystem functions need to be put into the specific context of the affected ecosystem. In the Mid-Atlantic region, most forest ecosystems

have resident earthworm communities primarily composed of naturalized European species. Any potential effects of *Amynthas* happen only after *Amynthas* successfully establishes itself and even expands its range within the forests. Competition between *Amynthas* and European species may take place during this process and the outcome will determine not only whether earthworm community structures will be changed but also whether any ecosystem function effects caused by *Amynthas* may take place. I addressed interspecific competition in **Chapter 3**.

Soil C dynamics is one of the major topics of ecosystem function research under the context of climate change and biological invasion. Soil respiration is the key process through which C is released from soil into the atmosphere as $CO_2$ gas, and has long been used as a proxy for soil C mineralization rates and an indicator of belowground activites. **Chapter 4** focuses on how *Amynthas* and interspecific interactions in earthworms affect soil respiration. I partitioned soil respiration into the litter C and soil C components, and addressed the underlying mechanisms that involve changes in microbial communities and activities.

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# 2. AN ILLUSTRATED KEY TO THE PHERETIMOID EARTHWORMS (*AMYNTHAS*, *METAPHIRE*, *PITHEMERA* AND *POLYPHERETIMA*) IN CONTINENTAL USA


## ABSTRACT

The invasion of the pheretimoid earthworms, including the genus *Amynthas*, in the United Sates has raised increasing concerns among ecologists and land managers, but the commonly used keys to this group are more than 30 years old and based primarily on internal morphology with outdated taxonomic information. The requirement of some significant amount of taxonomic expertise and dissection, even from the first entry of the key, keeps these keys from broader uses. As a result, many publications in the United States used *Amynthas* spp. to represent the group without identifying the exact species. I presented here a new key and diagnosis to the 15 pheretimoid earthworms, including 11 *Amynthas*, based on published records with modifications following inspecting specimens archived at the National Museum of Natural History, Washington, D.C. Photos of external characters were presented to help identification. A summary of current knowledge about the ecology and historical context was remarked for each species.


## INTRODUCTION

The pheretimoid earthworms, also variously referred to as *Pheretima* auct., the *Pheretima* group of genera or the *Pheretima* complex (Sims and Easton 1972; Chang et al. 2009; Blakemore 2010a), is a group of earthworms indigenous to Australia, Southeast

Asia, and the eastern part of East Asia. Previously in a single genus *Pheretima s.l.*, this group currently contains more than 1,000 species in 13-14 genera (Sims and Easton 1972; James 2004; Blakemore 2010b). In the last decade, the pheretimoids have become one of the most active fields of earthworm diversity research (Chang and Chen 2005; James 2004, 2009; Chang et al. 2008; Tsai et al. 2009, 2010; Blakemore 2010a, b; Hong and James 2011, 2013; Shen et al. 2013, 2014), with four new genera described in the last decade. Species from five genera, *Amynthas*, *Metaphire*, *Pheretima*, *Pithemera* and *Polypheretima*, have been widely reported invading temperate and tropical regions worldwide (Sims and Easton 1972; Easton 1981; Gates 1982; Blakemore 2010a; Chang et al. 2009). The first record of the pheretimoid earthworms in the continental United States is *Metaphire californica* Kinberg 1867 (as *Pheretima californica*) around San Francisco, CA. Since then, 16 species have been reported. These species belong to four genera, *Amynthas*, *Metaphire*, *Pithemera* and *Polypheretima*, including 11 species of *Amynthas*.

In recent years, invasion of some of the species, especially *A. agrestis*, *A. corticis*, *A. gracilis* and *A. hilgendorfi* into deciduous forests in eastern US has raised concerns regarding how these new invaders may affect plant communities, native soil fauna, and C and N biogeochemistry (Burtelow et al. 1998; Callaham et al. 2003; Snyder et al. 2009, 2011, 2013; Greiner et al. 2012). On the level of interactions between individual species, one species, *A. agrestis*, has been shown to compete with native millipedes for food resources, and may reduce species richness and diversity in the latter (Snyder et al. 2011, 2013). However, many of the forests invaded by *Amynthas* have already been colonized by naturalized European lumbricids earthworms of the genera *Lumbricus*, *Aporrectodea*

and *Octolasion*. Therefore, the impacts of the Asian earthworms will depend on how earthworm community structures are affected by *Amynthas*, and whether the earthworm communities containing or even dominated by *Amynthas* function differently from the communities primarily composed of European species. In forests already dominated by European species, interspecific competition as a result of *Amynthas* invasion may lead to changes in earthworm community structures. Competition for leaf litter was demonstrated between *A. hilgendorfi* and one of the most common European earthworm *Lumbricus rubellus*, with *A. hilgendorfi* being the superior competitor (see Chapter 3). The same European species also suffered reduced biomass with the presence of *A. agrestis* (Zhang et al. 2010). However, whether any potential *Amynthas*-induced changes in earthworm community structures will affect ecosystem functions still remains unclear. Recent studies seem to show that the effects of *A. agrestis*, *A. corticis*, *A. gracilis* and *A. hilgendorfi* on several ecosystem functions are similar and comparable to those induced by European species (Burtelow et al. 1998; Snyder et al. 2009, 2011; Greiner et al. 2012; Zhang et al. 2013), but there is no evidence suggesting that the mechanisms and/or pathways leading to these effects are the same between *Amynthas* and European lumbricids species.

Checklists of North American earthworm taxa have been provided by Gates (1982) and recently by Reynolds and Wetzel (2004) and Blakemore (2006). The current lists by Reynolds and Wetzel and by Blakemore list 161 and183 species respectively, of which 116 and 123, respectively, are native. Although the list of the pheretimoid earthworms in the United States have not changed dramatically after Gates (1982), our knowledge about the taxonomy and synonyms of the recorded species have evolved gradually. Several

names listed by Gates (1982) and subsequently by Reynolds and Wetzel (2004) have been regarded as junior synonyms, and their use should be strongly discouraged: *A. diffringens* (a junior synonym of *A. corticis*), *A. hawayanus* (*A. gracilis*), and *M. levis* (*A. tokioensis*).

Major challenges of species identification in this group include understanding the range of morphological variations of a species, which is usually spread across several taxonomic publications and further complicated by parthenogenetic degradations. These challenges are further exacerbated by the lack of a user-friendly key specifically designed for US species. The comprehensive key to cosmopolitan pheretimoid earthworms by Blakemore (2010a) is a useful primary resource for identifying peregrine species in general, but it contains too many species that have not been recorded in the US. The available keys to US pheretimoid species by Reynolds (1978) and Gates (1982) are outdated and start with the presence or absence of caeca, and therefore require users to dissect the specimens from the onset of using the keys. In fact, distinction and identification of mature specimens of the 16 species recorded in the US is usually, if not always, possible without dissection, although internal morphology is helpful for further confirming the identification.

In this study, I present a new key to US pheretimoid earthworms with photos and diagnosis. The key was designed to enable species identification without dissecting specimens, but at the same time internal morphologies were still included in the key to help double-checking the identification or to deal with situations in which external structures are poorly preserved due to non-optimal specimen processing, fixation or

preservation. The photos were based on specimens achieved at the National Museum of Natural History, Smithsonian Institution, Washington D.C., USA.

The purpose of this work is not to serve as the taxonomic authority of the species involved, but to provide a "field guide" to ecologists and educators whose work requires them to identify these species. We follow the species list by Blakemore (2006) with the exception of provisionally retaining *A. hilgendorfi* in the genus *Amynthas* as opposed to transferring it to *Metaphire* based on our knowledge about the close phylogenetic relationships among *A. hilgendorfi*, *A. agrestis* and *A. tokioensis*. One related issue is whether a species should belong to *Metaphire* or *Amynthas* (e.g. Chang and Chen 2004, 2005). This debate has been well documented (James 2005, James et al. 2005, Blakemore 2010a, 2012, Chang et al. 2009, 2014) and I will not get into it. While I followed the convention of listing synonyms from previous studies under each species, we restricted our lists to only publications that report US records or have significant taxonomic importance. The lists were not meant to be exhaustive, but to provide enough information on the name changes and synonyms that researchers working on US pheretimoid earthworms are most likely to encounter. All morphological descriptions listed under the diagnosis of each species were compiled from published studies, which were explicitly listed under the "data source". In most cases, we include only original morphological descriptions in our data source and avoid publications which themselves are compilations of published work (e.g. Blakemore 2010a; Chang et al. 2009). However, we sometimes include the latter as our data source; in those cases, only the data that are original in those publications were included.

## GENERAL MORPHOLOGY AMONG AMERICAN PHERETIMOIDS

The following descriptions apply to both amphimictic and parthenogenetic species, but various degrees of degradation related to spermathecae and prostate glands can be found in the latter. The descriptions are not exhaustive; they mean to provide enough information to help understand and recognize the group. Readers are encouraged to look at Appendix A and publications by Gates (1972), Sims and Easton (1972) and Easton (1979, 1982) for more details.

Clitellum annular in XIV-XV, intersegmental furrow absent. Setae numerous, forming a complete equatorial circle around each segment (pericheatine), beginning on II. Spermathecal pores 1-5 pairs in 4/5-8/9. Female pore single in XIV, mid-ventral. Male pores one pair in XVIII, ventral, superficial or in copulatory pouches. Spermathecae 1-5 pairs in V-IX. Testes two pairs in X, XI (holandric). Gizzard single in VIII-IX. Prostate glands one pair in XVIII, racemose, extending anteriorly and posteriorly through several segments. Caeca present or absent; when present, one pair, originating from XXVII or XXII, usually extending anteriorly through 2-4 segments.

## KEY TO SPECIES

This key is designed for species and specific morphs recorded in continental US.

1a. Size of mature (clitellate) specimens 20-50 mm x 1.5-2 mm; color light red to reddish white; male pore large, simple, superficial ..... *Amynthas minimus*

1b. Size of mature (clitellate) specimen >50 mm ..... (2)

2a. Female pore closely paired; spermathecal pores five pairs in 4/5/6/7/8/9; caeca in XXII ..... *Pithemera bicincta*

2b. Female pore single; spermathecal pore four pairs or fewer; caeca in XXVII or absent ..... (3)

3a. Male pores absent on one or both sides ..... (4)

3b. Both male pores present, with large, widely paired genital markings in some or all of XVII-XXIV, slightly median to male pores (Fig. 2.1) ..... (5)

3c. Both male pores present, in invaginations/copulatory pouches with no apparent genital markings (Fig. 2.2) ..... (6)

3d. Both male pores present, superficial, genital markings present or absent, when present, not arranged as in 3b (Fig. 2.3) ..... (7)

4a. Spermathecal pores three pairs, with or without finely wrinkled or crosshatches epidermal modification on VII and/or VIII ..... *Amynthas agrestis* (part)

4b. Spermathecal pores two pairs or fewer, with mid-ventral, presetal clusters of small tubercles in some or all of VIII-IX ..... *Amynthas hilgendorfi* (part)

4c. Spermathecal pores two pairs or fewer with small discs adjacent to the pores ..... ..... *Amynthas tokioensis* (part)

4d. Spermathecal pores two pairs or fewer with no male pores and no genital markings .... ..... *Amynthas agrestis* (part), *Amynthas hilgendorfi* (part) or *Amynthas tokioensis* (part)

5a. Paired genital markings on 17/18 and 18/19; color green; caeca in XXVII ..... ..... *Amynthas hupeiensis*

5b. Paired genital markings on 17/18 and/or 18/19; spermathecal pores dorsal, four pairs in 5/6/7/8/9; preclitellar genital markings absent; caeca in XXVI ..... ..... *Amynthas rodericensis*

5c. Paired genital markings on setal line on XVII and XIX, male pore in invaginations; color brown ..... *Metaphire posthuma*

5d. Paired presetal genital markings on some or all of XIX-XXI; color light grey with pink or red anterior ..... *Polypheretima elongata*

6a. Spermathecal pores two pairs in 7/8/9 with no prelitellar genital markings; 1st dorsal pores in 11/12 or 12/13; opening of copulatory pouches transversely slit-like with notched edges; color red ..... *Metaphire californica*

6b. Spermathecal pores three pairs or fewer in 6/7/8/9, sometimes with genital markings next to the pores; 1st dorsal pore in 9/10 or 10/11; opening of copulatory pouches C-shaped ..... *Metaphire houlleti*

7a. Spermathecal pores four pairs in 5/6/7/8/9, prelitellar genital markings present ..... ..... *Amynthas corticis*

7b. Spermathecal pores three pairs or fewer ..... (8)

8a. Spermathecal pores three pairs in 5/6/7/8; postclitellar genital markings present in XVIII, paired postsetal small discs median to male pores in tight clusters of 1-11; color red or light red ..... *Amynthas gracilis*

8b. Spermathecal pores two pairs or fewer in 6/7/8; male pore with 1-3 papillae, with one of them presetal median to the pore; caeca manicate ..... *Amynthas tokioensis*

8c. Spermathecal pores two pairs in 5/6/7; caeca simple ..... (9)

9a. Postciltellar genital markings absent outside the male pore area surrounded by circular folds (usually two papillae present within the area); each spermathecal pore associated with two papillae, one immediately in front of and one immediately behind each spermathecal pore, with the latter more median to the pore ..... ..... *Amynthas loveridgei*

9b. Postciltellar genital markings absent or present outside the male pore area surrounded by circular folds (usually two papillae present within the area), when present, presetal on XVIII and/or XIX; preclitellar genital markings present, highly variable but never like those in 9a ..... *Amynthas morrisi*

**DIAGNOSIS AND REMARKS**

**1. *Amynthas agrestis* (Goto & Hatai, 1899)** Fig. 2.4

*Perichaeta agrestis* Goto & Hatai, 1899: 17, 24.

*Pheretima agrestis* - Howell, 1939: 231. - Gates, 1953: 5; 1954: 224; 1958: 1, 31; 1963: 11; 1982: 38.

*Amynthas agrestis* - Sims and Easton, 1972: 235. - Reynolds, 1978: 119, 127; 2010: 143; 2011: 269. - Reynolds & Wetzel, 2004: 88; 2008: 179. Blakemore, 2010a: 429; 2013b: 56, 57.

Data source

Goto and Hatai 1899; Gates 1953, 1954, 1982; Blakemore 2010a, 2013b.

Diagnosis

Size 70-160 mm x 5-8 mm. Segment numbers 63-110. Color of live specimens red. Male pores usually absent; when present, small, superficial. Postclitellar genital markings usually absent; when present, paired presetal on XVIII, circular. Spermathecal pores three pairs in 5/6/7/8 or variously missing. Preclitellar genital markings present or absent; when present, areas of slight ventral. epidermal modification on VII and/or VIII, occasionally on VI and IX, unpaired and median or symmatrically paired, forming setal gaps, epidermis finely wrinkled or crosshatched, sometimes darker in color in live specimens. Female pore single in XIV. First dorsal pore 12/13. Spermatehcae present or absent; when fully present, three pairs in VI-VIII, ducts shorter than ampulla; diverticulum longer than duct and ampulla combined. Prostate glands present or absent; when present, extending through some or all of XVI-XXIII, ducts in XVIII. Caeca paired in XXVII, manicate.

Remarks

*A. agrestis* is a common species in the US. The first record of this species in continental US was in 1939 in the Homewood campus of the Johns Hopkins University, Baltimore, Maryland (Gates 1954, 1982). That record was the second of *A. agrestis* outside Japan, where the species is native. This species has recently been re-confirmed to be abundant in Baltimore and been observed to co-occur with *A. hilgendorfi* and *A. tokioensis* (Chih-Han Chang, personal observation), two species morphologically similar

to *A. agrestis*. This latter observation suggests the potential high chance of mis-identification. Reproduction of *A. agrestis* is parthenogenetic. In field conditions, *A. agrestis*, an annual species, overwinters only as cocoons and the adults reproduce in summer and die by the end of fall (Callaham et al. 2003; Richardson et al. 2009; Gorres et al. 2014). However, in laboratory conditions, its adults can survive through November-February (Snyder et al. 2013; Ikeda et al. 2015). This species is epi-endogeic, and its successful invasion in US forests has been attributed to dietary flexibility (Zhang et al. 2010). It has been known to compete with native millipedes for food resources in southeastern US, particularly the fragmented, partially decomposed leaf litter (Snyder et al. 2011, 2013). Current practice of using commercial mulches for horticulture and landscaping may be helping the spreading of this invasive species (Belliturk et al. 2015).

**2. *Amynthas corticis* (Kinberg, 1867)** Fig. 2.5

*Pheretima diffringens* - Gates, 1937: 350; 1954: 227; 1958: 31; 1963: 12; 1982: 44.

*Amynthas corticus* - Sims and Easton, 1972: 235.

*Amynthas diffringens* - Sims and Easton, 1972: 235. - Reynolds, 1978: 120, 127; 2010: 144; 2011: 270. - Reynolds & Wetzel, 2004: 88; 2008: 179.

Data source

Gates 1937, 1972, 1982; Blakemore 2013a

Diagnosis

Size 45-170 mm x 3-6 mm. Segment numbers 79-121. Color of live specimens greenish brown. Male pores paired in XVIII, simple, on circular to oval porophores. Postclitellar genital markings present or absent, when present, 1-3, small, around each male porophore, confined within concentric circular folds. Spermathecal pores four pairs in 5/6/7/8/9. Preclitellar genital markings variably present, presetal and postsetal; the presetal ones widely paired or unpaired in VII-X, behind and median to spermathecal pores; the postsetal ones just in front of each spermathecal pore. Female pore single in XIV. First dorsal pore 10/11, 11/12, or 12/13. Spermatehcae four pairs in VI-IX, duct shorter than ampulla, diverticulum with an oval seminar chamber and a longer, slender stalk; stalked glands associated with external genital markings. Prostate glands absent, present or rudimentary, ducts usually present. Caeca paired in XXVII, simple, extending anteriorly to XXII.

Remarks.

Historically frequently referred to as *Pheretima diffringens* or *Amynthas diffringens*, *A. corticis* is a common species in the US. The first record of this species in continental US was in 1866 in San Francisco, California, although the specimen was originally mis-identified as part of "*Pheretima californica*" (Gates 1973, 1954, 1982). Reproduction of *A. corticis* is usually parthenogenetic. *A. corticis*, an epi-endogeic species, has been reported to require both organic and mineral soil for optimal growth (Garcia and Fragoso 2002) and to compete directly with native millipedes for food resources in the southern Appalachian Mountains (Snyder et al. 2009).

**3. *Amynthas gracilis* (Kinberg, 1867)** Fig. 2.6

*Pheretima hawayana* - Gates, 1937: 354; 1954: 229; 1958: 31; 1963: 13; 1982: 47.

*Amynthas gracilis* - Sims and Easton, 1972: 235.

*Amynthas hawayanus* - Sims and Easton, 1972: 235. - Reynolds, 1978: 121, 127; 2010: 145; 2011: 271. - Reynolds & Wetzel, 2004: 88; 2008: 179.

Data source

Gates 1937, 1972, 1982; Blakemore 2013a.

Diagnosis

Size 56-156 mm x 3-6 mm. Segment numbers 70-101. Color of live specimens red. Male pores paired in XVIII, small, superficial, on a small porophore. Postclitellar genital markings present or absent; when present, paired postsetal small discs median to male pores, in tight clusters of 1-11. Spermathecal pores three pairs in 5/6/7/8. Preclitellar genital markings present or absent; when present, paired postsetal small discs median to spermathecal pores in some of VI-IX. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermatehcae three pairs in VI-VIII, duct slender, as long as or shorter than ampulla, with a tubular diverticulum shoter than the main axis. Prostate glands well developed, extending through some or all of XVI-XXIV. Caeca paired in XVII, simple, extending anteriorly to XXIV.

Remarks

Historically frequently referred to as *Pheretima hawayana* or *Amynthas hawayanus*, *A. gracilis* is a common species in the US. The first record of this species in continental

US was in 1905 in Illinois (Gates 1937, 1954, 1982). Reproduction of *A. gracilis* is amphimictic. *A. gracilis*, an epi-endogeic species, has been suggested to increase C and N flux in forest soils in northeastern US and may have potential long-term impacts on nutrient cycling (Burtelow et al. 1998).

**4. *Amynthas hilgendorfi* (Michaelsen, 1892)** Fig. 2.7

*Pheretima hilgendorfi* - Gates, 1954: 230; 1958: 11, 31; 1982: 49.

*Amynthas hilgendorfi* - Sims and Easton, 1972: 235, 237. - Reynolds, 1978: 122, 127; 2010: 146; 2011: 272. - Reynolds & Wetzel, 2004: 88; 2008: 179.

*Metaphire hilgendorfi* - Blakemore, 2010a: 416; 2012b: 106, 108; 2013b: 61.

Data source

Gates 1954, 1982; Blakemore 2010a, 2013b

Diagnosis

Size 109-170 mm x 6-8 mm. Segment numbers 98-118. Color of live specimens red or reddish brown. Male pores usually absent; when fully present, in invaginations. Postclitellar genital markings present or absent; when present, similar to those in the preclitellum region, in XVII-XVIII, occasionally in XIX-XXII. Spermathecal pores two pairs in 6/7/8. Preclitellar genital markings unpaired, mid-ventral, presetal clusters of numerous small tubercles in VIII-IX, occasionally in VII, X, XI. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermatehcae two pairs in VII-VIII, large, duct shorter than ampulla, with a diverticulum longer than the main axis; genital marking

glands present with long coelomic stalks. Prostate glands present or absent; when present, large in XV-XXI. Caeca paired in XXVII, manicate, extending anteriorly to XXII, XXIII or XXIV.

Remarks

A common species in the US, *A. hilgendorfi* was first recorded in 1948 in Kingston in New York (Gates 1954). Reproduction of this species is obligatory parthenogenetic and almost all US specimens reported so far lack male pores. However, an NMNH specimen identified by G.E. Gates has a male pore in the form of an everted copulatory pouch on one side, supporting potential transferring to *Metaphire* (Blakemore 2010a, 2013b). *A. hilgendorfi* is an epi-endogeic species. Its signature granular casts can be seen on the soil surface and sometimes it turns the top layer of several centimeters of soil into casts under high activity and abundance. This species is common in urban and suburban areas and is also invading deciduous forests in eastern US. Its spreading is probably facilitated by commercial mulches, as is the case in *A. agrestis*. It has been shown to be the superior competitor when compete with *Lumbricus rubellus*, a non-native, epi-endogeic European earthworm common in the US, for leaf litter and may potentially outcompete the latter (see Chapter 3).

**5. *Amynthas hupeiensis* (Michaelsen, 1895)** Fig. 2.8

*Pheretima hupeiensis* - Gates, 1937: 356; 1954: 234; 1958: 17, 31; 1963: 13; 1982: 52. *Amynthas hupeiensis* - Sims and Easton, 1972: 237. - Reynolds, 1978: 123, 127; 2010: 147; 2011: 273. - Reynolds & Wetzel, 2004: 88; 2008: 179.

Data source

Gates 1937, 1954, 1958, 1982

Diagnosis

Size 40-220 mm by 3-6 mm. Segment numbers 97-138 mm. Color of live specimens green. Male pores paired in XVIII, on a small circular porophore. Postclitellar genital markings two pairs, one on 17/18 and one on 18/19, slightly median to the male pores. Spermathecal pores three pairs on the anterior margin of VII-IX. Preclitellar genital markings absent. Female pore single on XIV. First dorsal pore 11/12 or 12/13. Spermatehcae three pairs in VII-IX, duct shorter than ampulla; diverticulum longer than main axis, with a stalk shorter than the elongate tubular seminal chamber. Prostate glands paired, medium size, extending through XVI, XVII to XIX, XX. Caeca paired in XVII, simple, extending anteriorly to XXIV.

Remarks

A common species in the US, *A. hupeiensis* was first recorded from specimens collected in 1910 in Washington D.C. While this species probably originated from China, Gates (1982) believed that it came to the US from Japan with the flowering cherry trees currently featuring the "Cherry Blossom Festival" in the national's capital. All American specimens reported so far are parthenogenetic, but some earlier specimens were claimed to be amphimictic (Gates 1982). *A. hupeiensis* seems to prefer sandy soils and is one of the species found along sandy river banks. The density of this species can reach at least

110 individuals/$m^2$, and its casts have caused problems in golf courses (Gates 1982; Redmond et al. 2014), in which the species is considered a pest.

**6. *Amynthas loveridgei* (Gates, 1968)** Fig. 2.9

*Pheretima loveridgei* Gates, 1968: 257; 1982: 57.

*Amynthas loveridgei* – Sims and Easton, 1972: 236. – Reynolds, 1978: 127; 2011: 274. – Reynolds & Wetzel, 2004: 88; 2008: 180. – Blakemore, 2014: 129, 130.

Data source

Gates 1968, 1982; Blakemore 2014; present study.

Diagnosis

Size 90-113 mm x 4-6 mm. Segment numbers 118-169. Color of live specimens unknown. Male pores absent or not identifiable. Postclitellar genital markings three tubercles on each side on XVIII, triangularly arranged, one lateral, one presetal, one postsetal, surrounded by circular folds. Spermathecal pores two pairs in 5/6/7. Preclitellar genital markings present, one in front of and one behind each spermathecal pore, the latter more median to the pore. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermatehcae two pairs in VI-VII, ducts as long as ampulla; diverticulum small or degenerated, usually shorter than the main axis. Prostate glands absent or well developed; when present, extending through XVI, XVII to XXIII, XXIV. Caeca paired in XXVII, simple, extending anteriorly to XXIII.

Remarks

One of the two pheretimoid earthworms described from the US, *A. loveridgei* was first recorded in 1966 (Gates 1968) in Greenville, Madison County, Florida; in the US this species has been reported so far only in Georgia and Florida. This species can be easily confused with *A. morrisi*, which has been found previously misidentified as *A. loveridgei* in the NMNH specimens (USNM 125048, Chih-Han Chang, personal observation). Reproduction of *A. loveridgei* is parthenogenetic.

**7. *Amynthas minimus* (Horst, 1893)** Fig. 2.10

*Pheretima minima* - Gates, 1982: 57.

*Amynthas minimus* - Sims and Easton, 1972: 236. - Reynolds, 1978: 127; 2011: 275. - Reynolds & Wetzel, 2004: 88; 2008: 180.

Data sources

Gates 1972, 1982; Chang et al. 2009 (for color of live specimens).

Diagnosis

Size 20-56 mm x 1.5-2 mm. Segment numbers 77-115. Color of live specimens light red to reddish white. Male pores paired in XVIII, on a relatively large porophore. Postclitellar genital markings unpaired, small tubercles, mid-ventral presetal on some of XVII, XIX-XXI. Spermathecal pores paired in 5/6 or absent. Preclitellar genital markings paired small presetal tubercles on some of V-VIII. Female pore single in XIV. First

dorsal pore 11/12 or 12/13. Spermatehcae present or absent; when present, one pair or single in VI, large. Prostate glands present or absent; when present, large, extending through some or all of XVI-XXIII. Caeca simple, extending anteriorly to XXIII.

Remarks

Easily distinguished from most pheretimoid species by its body size, *A. minimus* is one of the smallest pheretimoid earthworms in the world, and was first recorded in the US in 1969 in Louisiana (Gates 1982). Reproduction of this species is parthenogenetic.

**8. *Amynthas morrisi* (Beddard, 1892)** Fig. 2.11

*Pheretima morrisi* - Gates, 1937: 361; 1954: 238; 1958: 31; 1963: 14; 1968: 253; 1982: 60.

*Amynthas morrisi* - Sims and Easton, 1972: 236. - Reynolds, 1978: 127; 2010: 148; 2011: 276. - Reynolds & Wetzel, 2004: 88; 2008: 180. - Blakemore, 2014: 131.

Data sources

Gates 1937, 1968, 1972, 1982; Blakemore 2014.

Diagnosis

Size 40-150 mm x 2-6 mm. Segment numbers 75-102. Color of live specimens unknown. Male pores paired in XVIII, on small, circular porophores. Postclitellar genital markings present or absent; when present, two immediately median to each male

porophore, one presetal and one postsetal, with one of the two lacking possible, slightly larger than the porophore, surrounded several concentric furrows; additional presetal markings in XVIII and XIX in some specimens, mid-ventral or median to male pores, numbers variable. Spermathecal pores two pairs in 5/6/7. Preclitellar genital markings paired and/or unpaired small discs varying in numbers and positions; paired in some of VI-IX, median to spermathecal pore; unpaired in some of V-IX, mid-ventral, presetal. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermathecae two pairs in VI-VII. Prostate glands paired, extending through some or all of XVII-XXIII. Caeca paired in XVII, simple, extending anteriorly to XIV.

Remarks

*A. morrisi* was first recorded in continental US in1916 in Waxahachie, Texas (Gates 1937). The identity of what have been commonly recognized as "*A. morrisi*" in Asia and North America was recently questioned by Blakemore (2014). As the type specimen of *A. morrisi* is missing and the original description by Beddard lacks enough details, addressing this problem is unattainable in the present study. Nevertheless, all US specimens registered as *A. morrisi*/*Pheretima morrisi* in the NMNH collection appear to belong to a single species (Chih-Han Chang, personal observation). Reproduction of *A. morrisi* is amphimictic.

**9. *Amynthas rodericensis* (Grube, 1879)** Fig. 2.12

*Pheretima rodericensis* - Gates, 1954: 239; 1958: 31; 1963: 14; 1982: 63.

*Amynthas rodericensis* - Sims and Easton, 1972: 235. - Reynolds, 1978: 127; 2010: 149; 2011: 277. - Reynolds & Wetzel, 2004: 88; 2008: 180.

Data sources

Gates 1937, 1954, 1972, 1982

Diagnosis

Size 55-150 mm x 3-10 mm. Segment numbers 80-100. Color of live specimens unknown. Male pores piared in XVIII, small, sperficial, on small, circular porophores. Postclitellar genital markings paired, oval, large, median to male pores, across 17/18 and/or 18/19. Spermathecal pores four pairs in 5/6/7/8/9, dorsal. Preclitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermatehcae four pairs in VI-IX, duct shorter than ampulla; diverticulum stalks longer than the ducts, seminal chamber elongate, ellipsoidal, or moniliform. Prostate glands paired, extending through XVI, XVII to XXI, XXII. Caeca paired in XXVII, simple, extending anteriorly to XXIV-XXV.

Remarks

The first record of *A. rodericensis* in continental US was in 1950 in Lutz, Florida (Gates 1954). This species is the only US pheretimoid earthworms with dorsally positioned spermathecal pores. Identification of this species can easily be achieved by combining this unique spermathecal pore position and the genital markings in the male pore area. Reproduction of *A. rodericensis* is amphimictic.

**10. *Amynthas tokioensis* (Beddard, 1892)** Fig. 2.13

*Perichaeta levis* Goto and Hatai, 1899: 20.

*Pheretima levis* - Gates, 1954: 234; 1958: 21, 31; 1963: 14; 1982: 54.

*Metaphire levis* - Sims and Easton, 1972: 238. - Reynolds, 1978: 124, 127; 2010: 151; 2011: 280. - Reynolds & Wetzel, 2004: 88; 2008: 181.

*Amynthas tokioensis* - Sims and Easton, 1972: 237. - Blakemore, 2010a: 422.

*Amynthas levis* - Blakemore, 2012b: 111.

Data sources

Gates 1954, 1958, 1982; Blakemore 2010a, 2012b

Diagnosis

Size 75-125 mm x 5-7 mm. Segment numbers 84-102. Color of live specimens red. Male pores present or absent; when present, paired in XVIII, on a small porophore surrounded by a deep furrow. Postclitellar genital markings small, circular discs, 1-3 on each side of XVIII, one sometimes on the porophore, two median or lateral to the male porophore. Spermathecal pores two pairs in 6/7/8, variously lacking. Preclitellar genital markings small, circular discs, two associated with each spermathecal pore, one in front of and one behind, the posterior one median to the pore. Female pore single in XIV. First dorsal pore 12/13. Spermatehcae present or absent; when fully present, two pairs in VII-VIII, duct as long as or shorter than ampulla, diverticulum longer than the main axis, stalk as long as seminal chamber; 2-3 stalked accessory glands associated with each

spermatheca. Prostate glands paired, extending through some or all of XV-XXIII; three associate gland associated with each male pore. Caeca paired in XXVII, manicate.

Remarks

Historically frequently referred to as *Pheretima levis* or *Metaphire levis*, *A. tokioensis* is a common species in the US. The first record of this species in continental US was in 1947 in New York City (Gates 1954). Gates (1982) believed that it was probably brought to the US directly from Japan. *A. tokioensis* frequently co-occurs with *A. agrestis* and/or *A. hilgendorfi*, and is probably often overlooked partially due to difficulties in separating *A. tokioensis* from the other two species. Distinction among the three species is almost impossible when male pores and all genital markings are absent, as the three species all have manicate caeca and may bear two pairs or fewer spermathecal pores. Reproduction of *A. tokioensis* is parthenogenetic.

**11. *Metaphire californica* (Kinberg, 1867)** Fig. 2.14

*Pheretima californica* - Gates, 1937: 348; 1954: 226; 1958: 31; 1963: 12; 1982: 42. *Metaphire californica* - Sims and Easton, 1972: 238. - Reynolds, 1978: 127; 2010: 150; 2011: 278. - Reynolds & Wetzel, 2004: 88 ; 2008: 180.

Data sources

Gates 1937, 1972, 1982

Diagnosis

Size 50-132 mm x 3-5 mm. Segment numbers 82-115. Color of live specimens red. Male pores paired in XVIII, each in an invagination with transversely slit-like opening. Postclitellar genital markings absent. Spermathecal pores two pairs in 7/8/9. Preclitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermatehcae two pairs in VIII-IX, small to medium, ducts shorter than ampulla; diverticulum coiled or looped. Prostate glands present, extending through some or all of XVI-XXII. Caeca paired in XXVII, simple, extending anteriorly to XXII.

Remarks

One of the two pheretimoid earthworms described from the US, *M. californica* was first recorded in 1866 near San Francisco, California, and is also the first pheretimoid earthworm recorded in the US. Reproduction of this species amphimictic.

**12. *Metaphire houlleti* (Perrier, 1872)** Fig. 2.15

*Pheretima houlleti* - Gates, 1958: 31; 1982: 52.

*Metaphire houlleti* - Sims and Easton, 1972: 238. - Reynolds, 1978: 127; 2011: 279. - Reynolds & Wetzel, 2004: 88; 2008: 180.

Data sources

Gates 1937, 1972, 1982; Shen et al. 2005.

Diagnosis

Size 107-200 mm x 3-8 mm. Segment numbers 86-131. Color of live specimens unknown. Male pores paired in XVIII, each in a copulatory pouch with a C-shaped opening. Postclitellar genital markings two in each copulatory pouch. Spermathecal pores three pairs in 6/7/8/9, some or all missing in parthenogenetic morphs. Preclitellar genital markings absent or present; when present, within the intersegmental furrow, next to each spermathecal pore, one naterior, one posterior. Female pore single in XIV. First dorsal pore 9/10 or 10/11. Spermatehcae three pairs in VII-IX, some or all lacking in parthenogenetic morths, duct shorter than ampulla, diverticulum with short, slender stalk and wider, elongate, coiled seminal chamber; accessory gland stalked. Prostate glands paired, extending through some or all of XVI-XXIII. Caeca paired in XVII, simple, extending anteriorly to XXII-XXIV.

Remarks

*M. houlleti* was first recorded in continental US in 1969 in Georgia and Florida (Gates 1982). Both amphimictic and parthenogenetic have been reported in this species.

**13. *Metaphire posthuma* (Vaillant, 1868)** Fig. 2.16

*Pheretima posthuma* - Gates, 1958: 31; 1982: 61.

*Metaphire posthuma* - Sims and Easton, 1972: 239. - Reynolds & Wetzel, 2004: 88; 2008: 181. - Reynolds, 2011: 281.

Data sources

Gates 1937, 1972, 1982; Tsai 1964.

Diagnosis

Size 60-210 mm x 3-8 mm. Segment numbers 91-140. Color of live specimens brown.

Male pores paired in XVIII, in an invagination. Postclitellar genital markings paired on setal circle on XVII, XIX, slightly median to male pores. Spermathecal pores four pairs, on the posterior margin of segments, just in front of 5/6/7/8/9. Preclitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermatehcae four pairs in VI-IX, each with a short, stout duct and oval or heart-shaped ampulla; diverticulum stalk short and slender, seminal chambers usually longer than stalks. Prostate glands paired, extending through some or all of XV-XXI; accessory glands in XVII and XIX, corresponding to external genital markings. Caeca paired in XVII, simple, extending anteriorly to XIV.

Remarks

The first record of *M. posthuma* in continental US was in 1967 in Homestead, Florida (Gates 1982). Reproduction of this species is amphimictic.

**14. *Pithemera bicincta* (Perrier, 1875)** Fig. 2.17

*Pheretima bicincta* - Gates, 1958: 31; 1963: 12; 1982: 40.

*Pithemera bicincta* – Sims and Easton, 1972: 202, 232; Reynolds & Wetzel, 2004: 88; 2008: 181. - Reynolds, 2010: 152.

*Pithemira bicincta* - Reynolds, 2011: 283.

Data sources

Gates 1937, 1972, 1982

Diagnosis

Size 40-80 mm x 2-3 mm. Segment numbers 77-101. Color of live specimens red. Male pores paired in XVIII, small, on a porophore with variable size and shape. Postclitellar genital markings present or absent; when present, paired in XVIII or on 18/19. Spermathecal pores five pairs in 4/5/6/7/8/9. Preclitellar genital markings absent. Female pore closely paired in XIV. First dorsal pore 12/13. Spermatehcae present or absent; when present, five pairs in V-IX, with a duct as long as the ampulla; diverticulum usually shorter than duct and ampulla combined. Prostate glands present or absent; when present, medium to large, extending through some or all of XVI-XX, with a duct in XVIII. Caeca paired in XXII, simple, sometimes extending to XXI.

Remarks

*Pithemera bicincta* was first recorded in continental US in 1963 in a greenhouse in Maine (Gates 1963, 1982). Outside greenhouses, it has only been reported in Georgia and Florida. Reproduction of *Pithemera bicincta* is likely to be parthenogenetic.

**15. *Polypheretima elongata* (Perrier, 1872)** Fig. 2.18

*Pheretima elongata* - Gates, 1958: 31; 1982: 45.

*Metapheretima elongata* - Reynolds & Wetzel, 2004: 88; 2008: 181.

*Polypheretima elongata* - Easton, 1979: 53.

Data sources

Gates 1937, 1972, 1982; Chang et al. 2009

Diagnosis

Size 75-355 mm x 3-6 mm. Segment numbers 136-297. Color of live specimens light grey with pink to red anterior. Male pores paired in XVIII, on small disc. Postclitellar genital markings widely paired in some or all of XIX-XXIV, presetal, median to male pores. Spermathecal pores present or absent; when present, in some or all of 5/6/7, paired groups of 2-5. Preclitellar genital markings absent. Female pore single in XIV. First dorsal pore 12/13. Spermatehcae present or absent; when present, in some or all of VI-VII, with a duct shorter than ampulla, diverticulum present, genital marking glands sessile. Prostate glands XVI-XXI. Caeca absent.

Remarks

The only record of *Polypheretima elongata* in continental US was in a PhD thesis in 1969 from Louisiana (Gates 1958, 1982). Whether this species was actually present in the US is questionable. *Polypheretima elongata* was listed in the checklists by Gates (1982) and by Reynolds and Wetzel (2008). However, it was not reported in the Louisiana earthworm study by Reynolds (2008), and was not listed in the checklists by Reynolds and Wetzel (2004) and by Reynolds (2011), either. Both amphimictic and parthenogenetic have been reported in *Polypheretima elongata*.

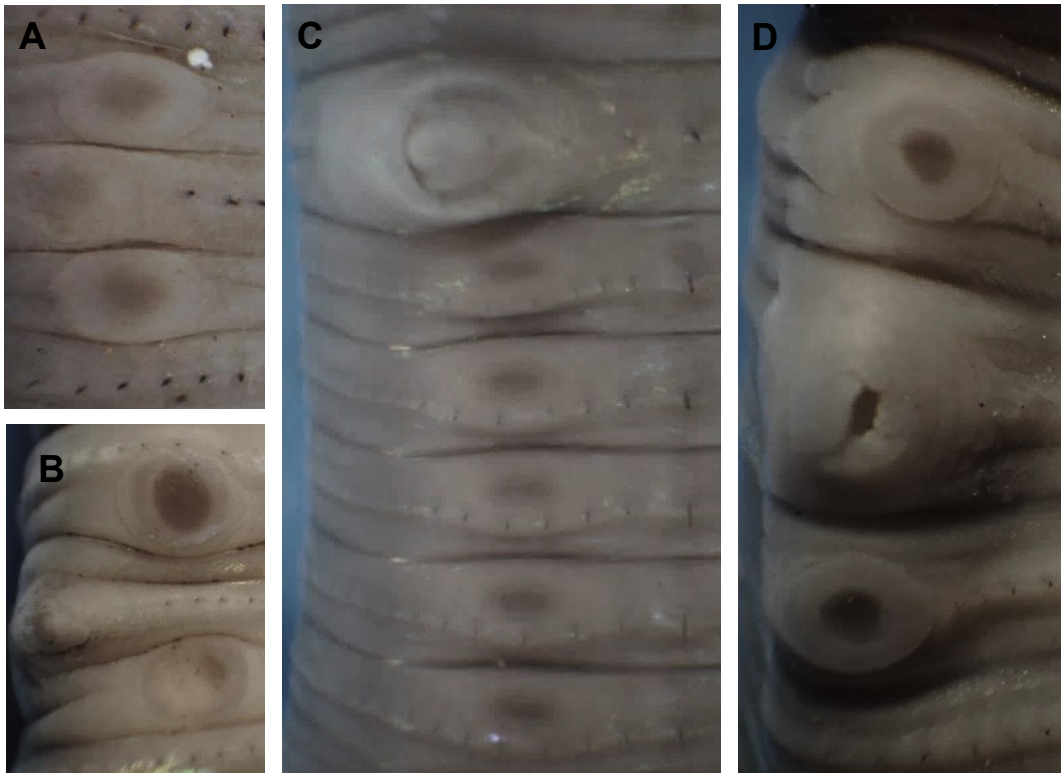


**FIG. 2.1.** Male pores with large genital markings in some or all of XVII-XXIV. (A) *Amynthas hupeiensis*. (B) *Amynthas rodericensis*. (C) *Polypheretima elongata*. (D) *Metaphire posthuma*.

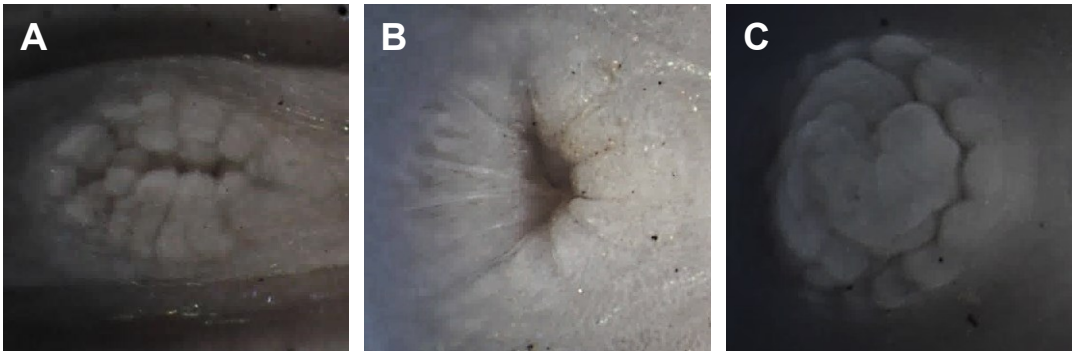


**FIG. 2.2.** Male pores in invaginations/copulatory pouches with no apparent genital markings. (A) *Metaphire californica*; not everted. (B, C) *Metaphire houlleti*; not everted (B) or everted (C).

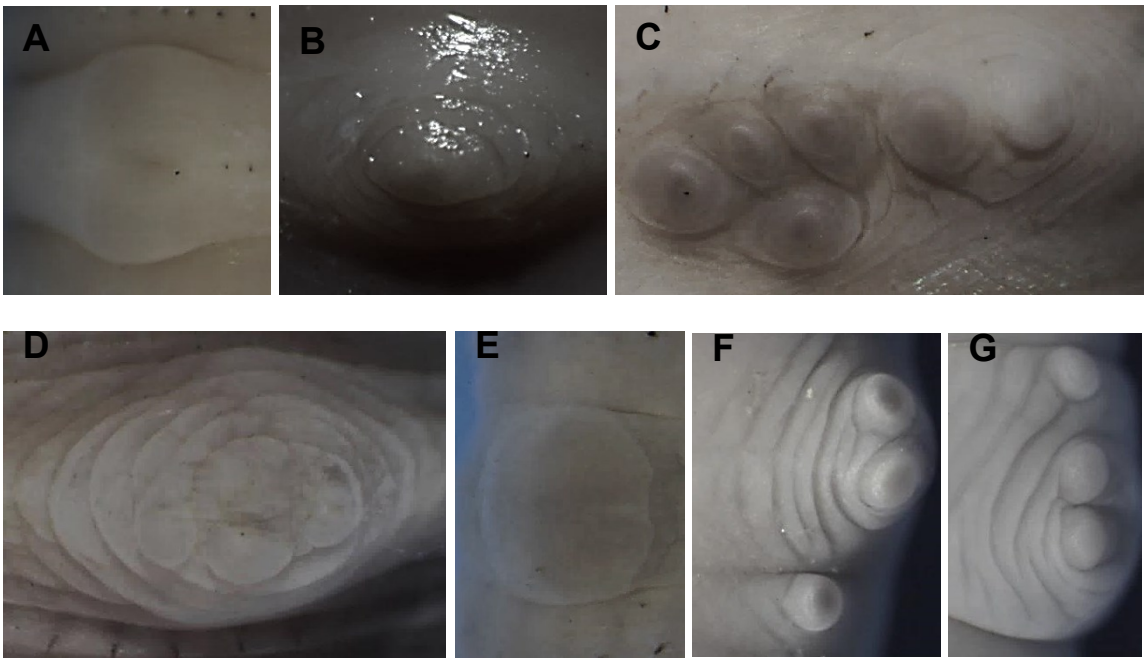


**FIG. 2.3.** Male pores not illustrated in Figs. 1 and 2. (A) *Pithemera bicincta*. (B) *Amynthas corticis*. (C) *Amynthas gracilis*. (D) *Amynthas loveridgei*. (E) *Amynthas minimus*. (F, G) *Amynthas morrisi*.

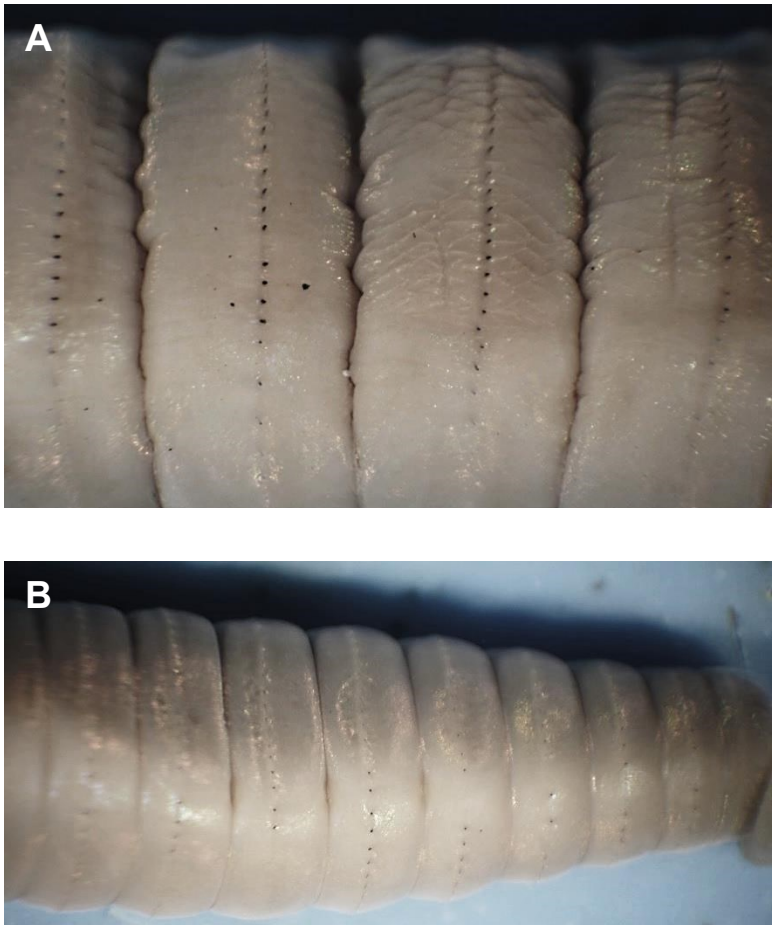


**FIG. 2.4.** *Amynthas agrestis*. (A) Two segments with wrinkled surface on the right and two segments of normal surface on the left. (B) Three spermathecal pores in the intersegmental furrows.

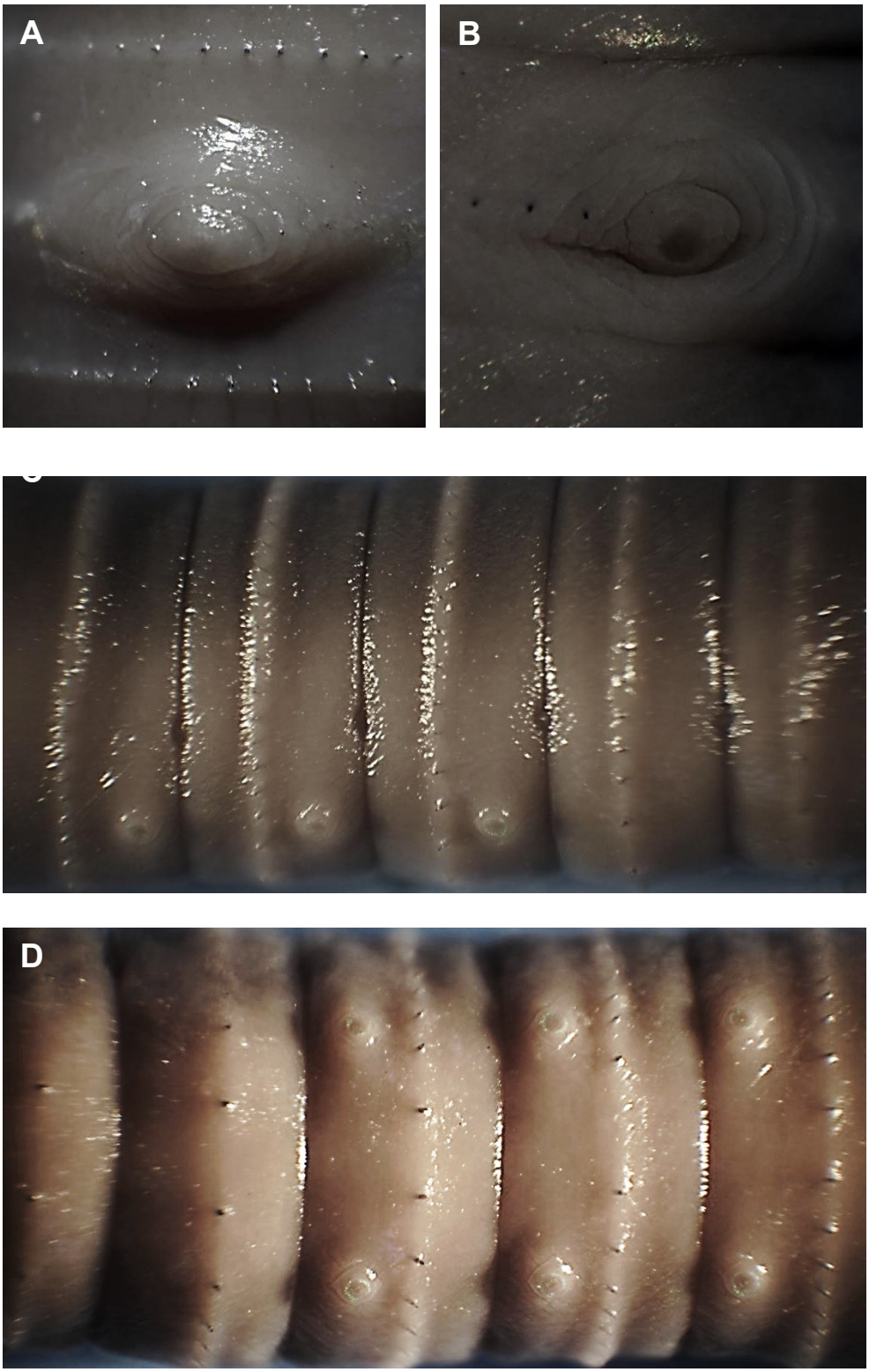


**FIG. 2.5.** *Amynthas corticis*. (A, B) Male pores. (C) Four spermathecal pores and genital markings; side view. (D) Preclitellum genital markings; ventral view.

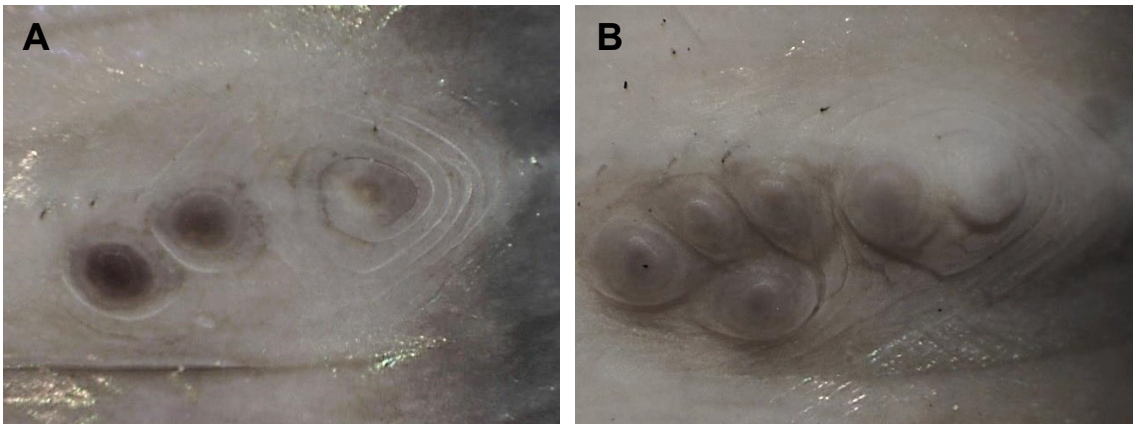


**FIG. 2.6.** *Amynthas gracilis*. (A, B) Male pores and the associated postclitellum genital markings.

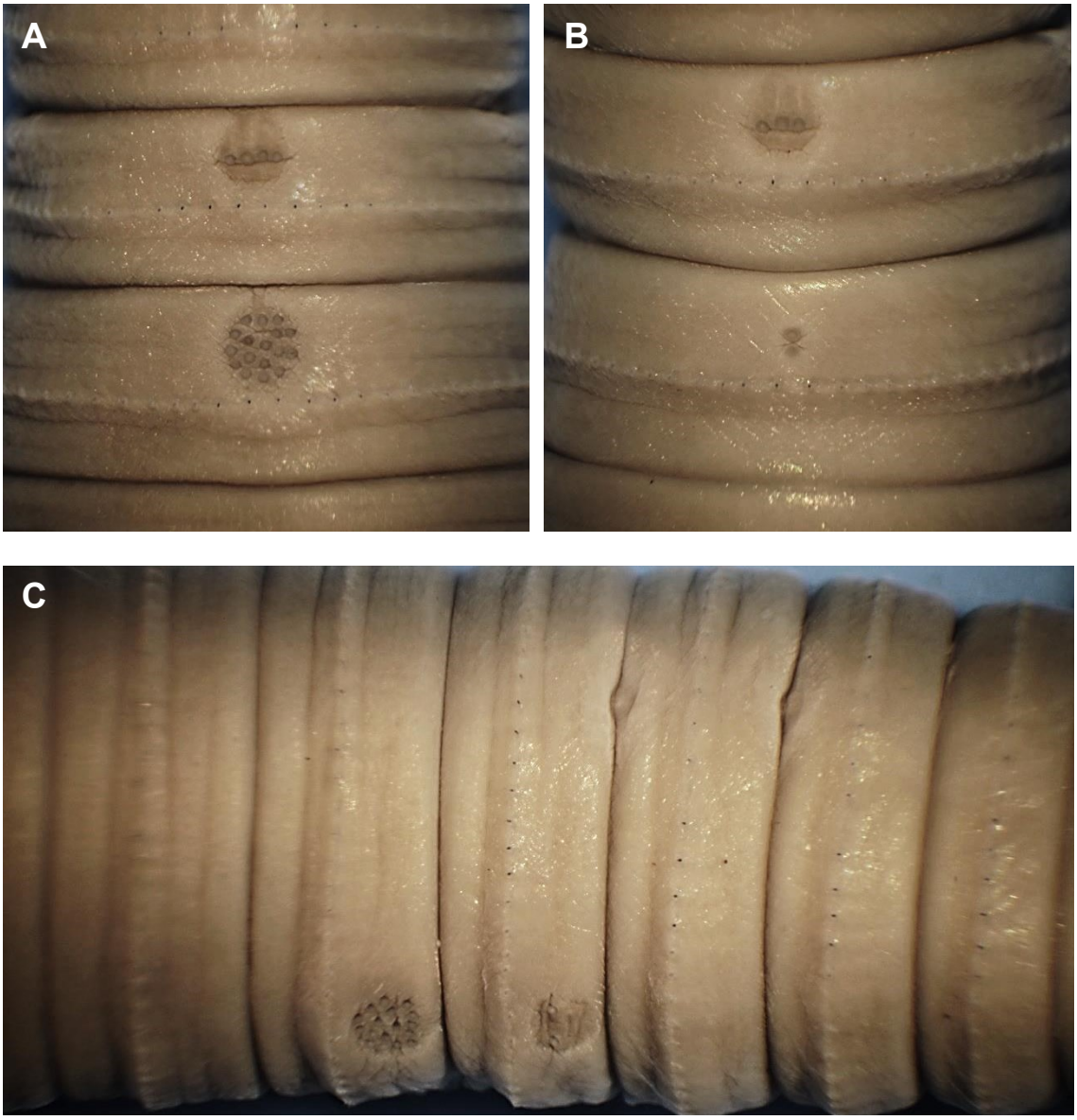


**FIG. 2.7.** *Amynthas hilgendorfi*. (A, B) Preclitellum genital markings, ventral view. (C) Two spermathecal pores and preclitellum genital markings, lateral view.

**FIG. 2.8.** *Amynthas hupeiensis*. Male pores and the associated postclitellum genital markings; ventral view.

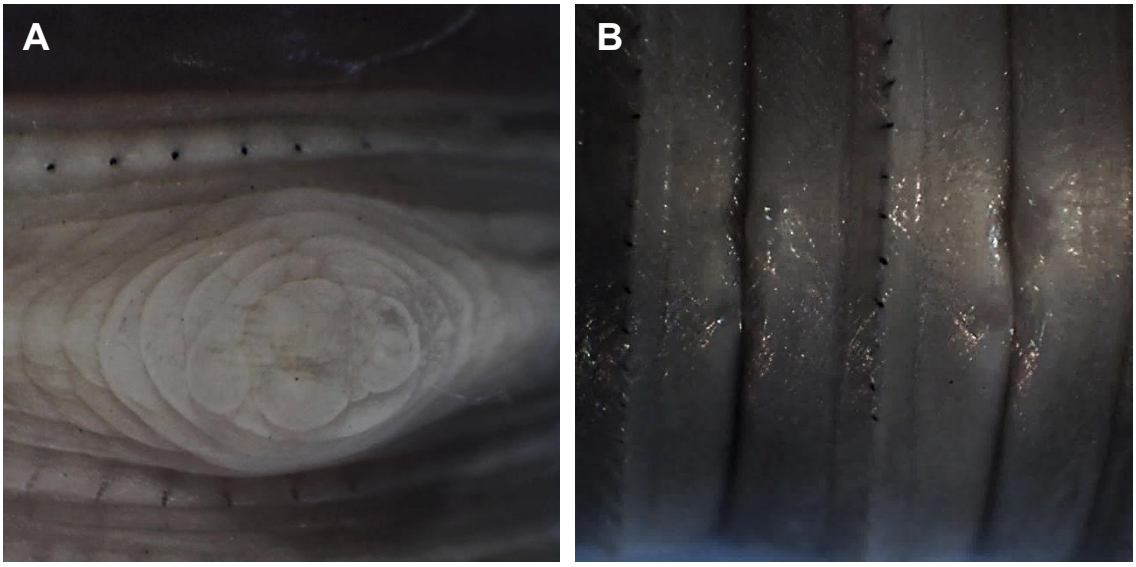


**FIG. 2.9.** *Amynthas loveridgei*. (A) Male pore. (B) Two spermathecal pores and the associated genital markings.

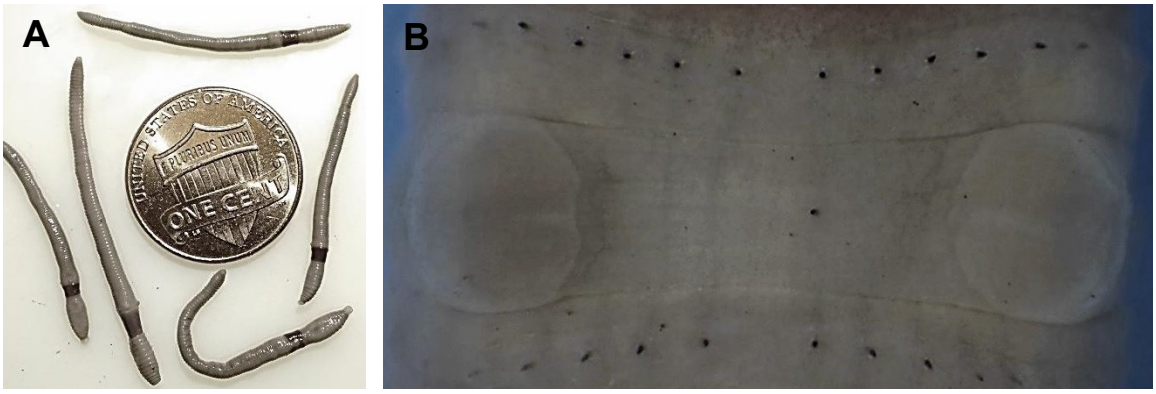


**FIG. 2.10.** *Amynthas minimus*. (A) Sizes compared to a one cent coin. (B) Male pores; ventral view.

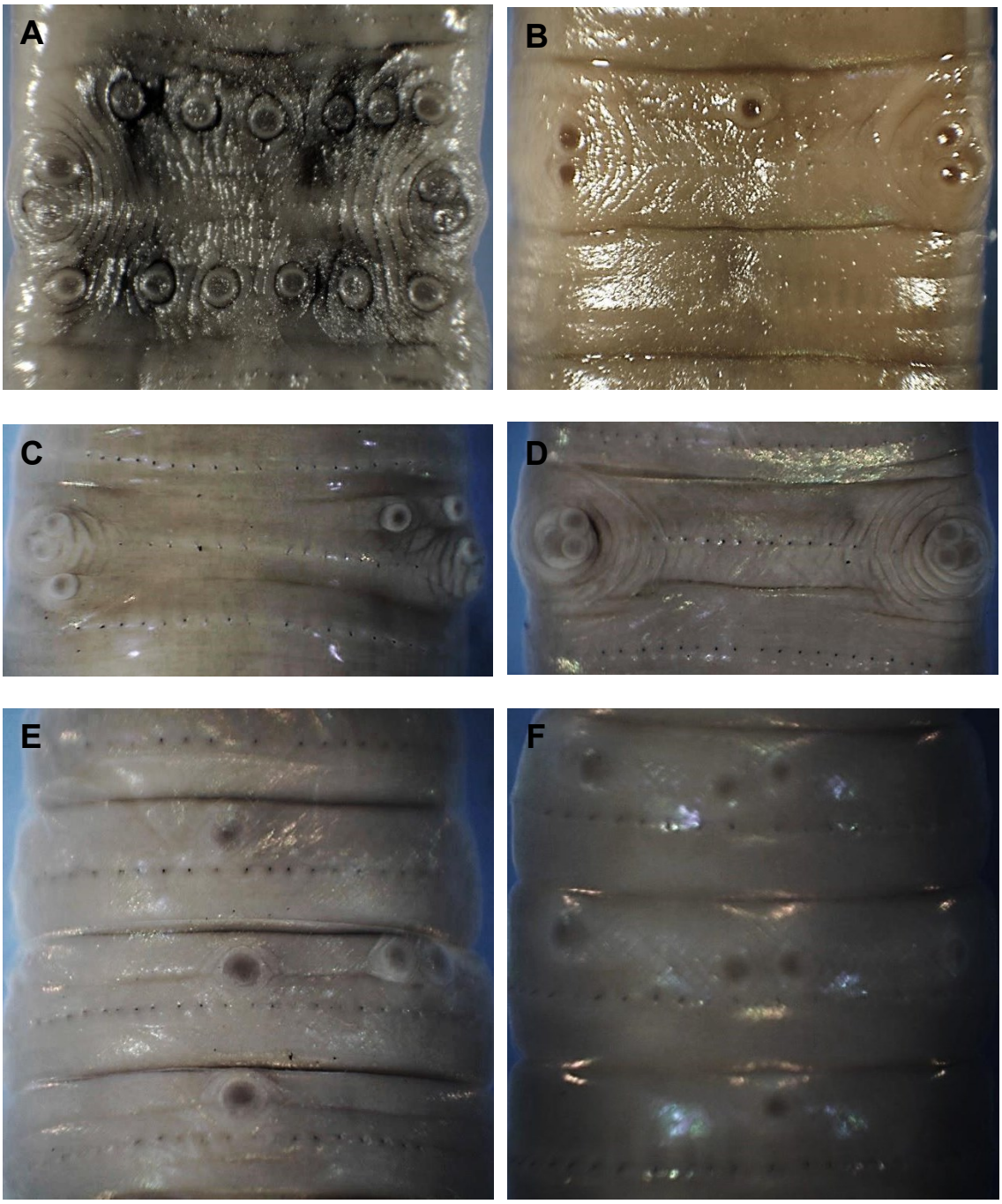


**FIG. 2.11.** *Amynthas morrisi*. (A-D) Male pores with various numbers and positions of postclitellum genital markings. (E, F) Preclitellum genital markings.

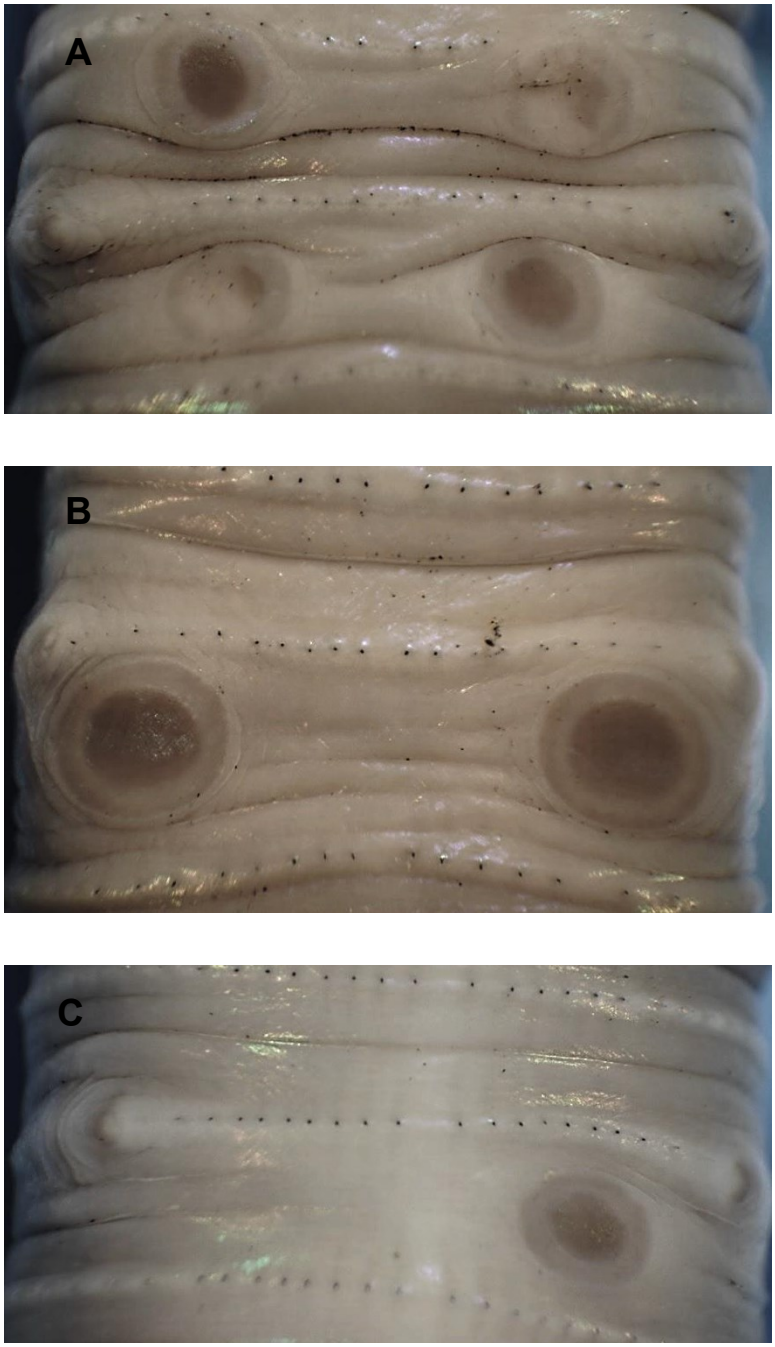


**FIG. 2.12.** *Amynthas rodericensis*. (A-C) Male pores and the associated genital markings.

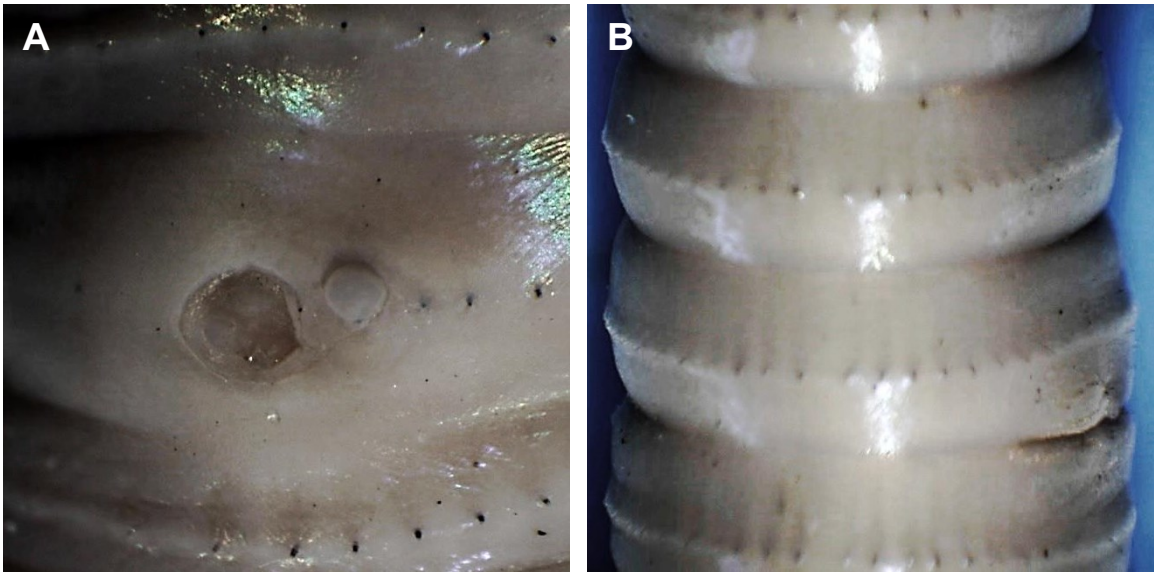


**FIG. 2.13.** *Amynthas tokioensis*. (A) Male pore and the associated genital marking. (B) Single spermathecal pore in the intersegmental furrow on the lower right, compared to its absence on the lower left and in other intersegmental furrows.

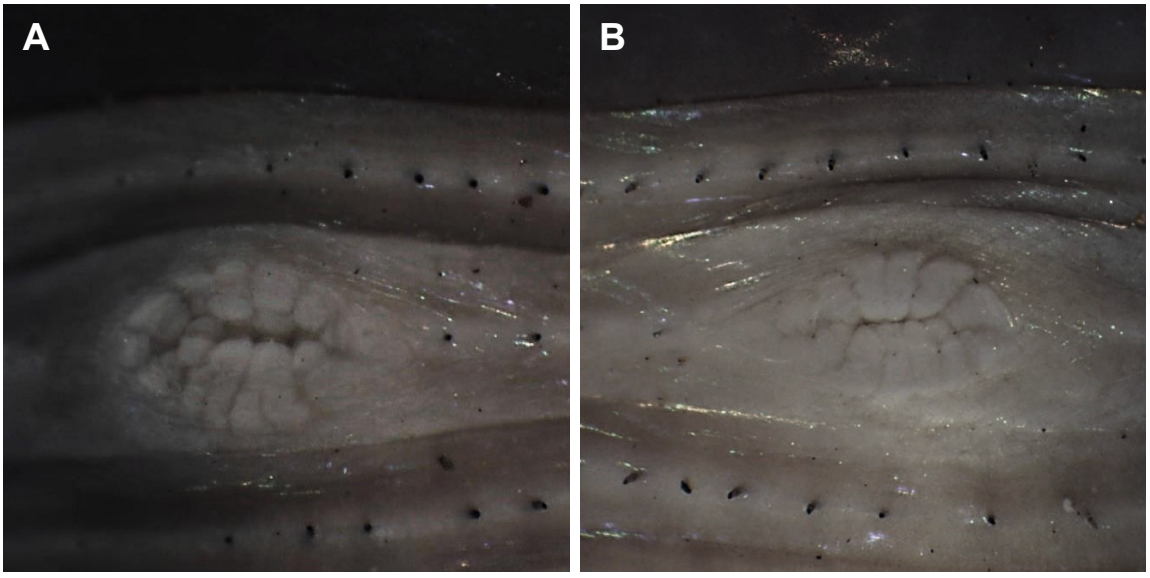


**FIG. 2.14.** *Metaphire californica*. (A, B) Male pores inside copulatory pouches (invaginations).

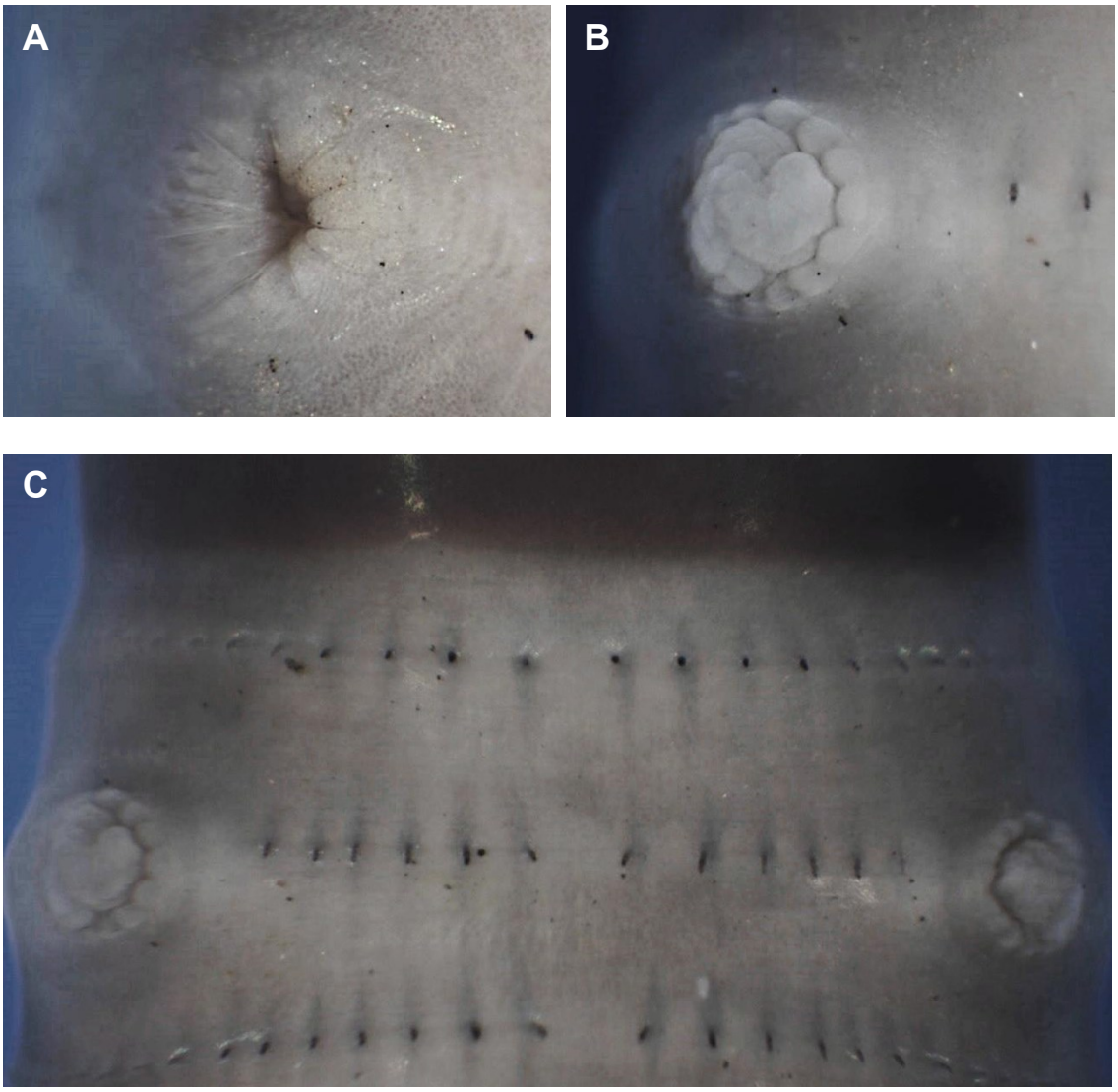


**FIG. 2.15.** *Metaphire houlleti*. (A) Male pore inside a populatory pouch. (B) Male pore with copulatory pouch everted. (C) Male pores; ventral view.

**FIG. 2.16.** *Metaphire posthuma*. Male pores and the associated genital markings.

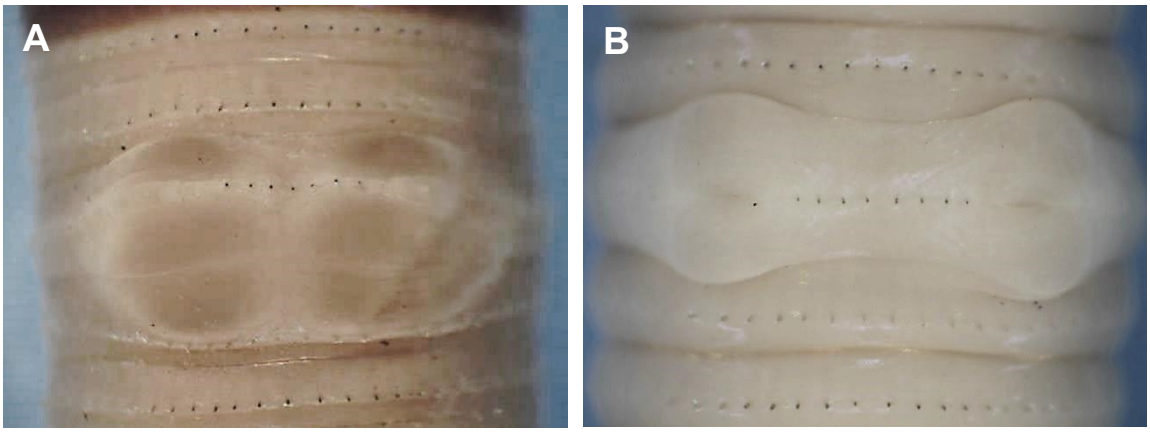


**FIG. 2.17.** *Pithemera bicincta*. (A, B) Male pores and the associated genital markings of two different specimens, showing potential intraspecific variations.

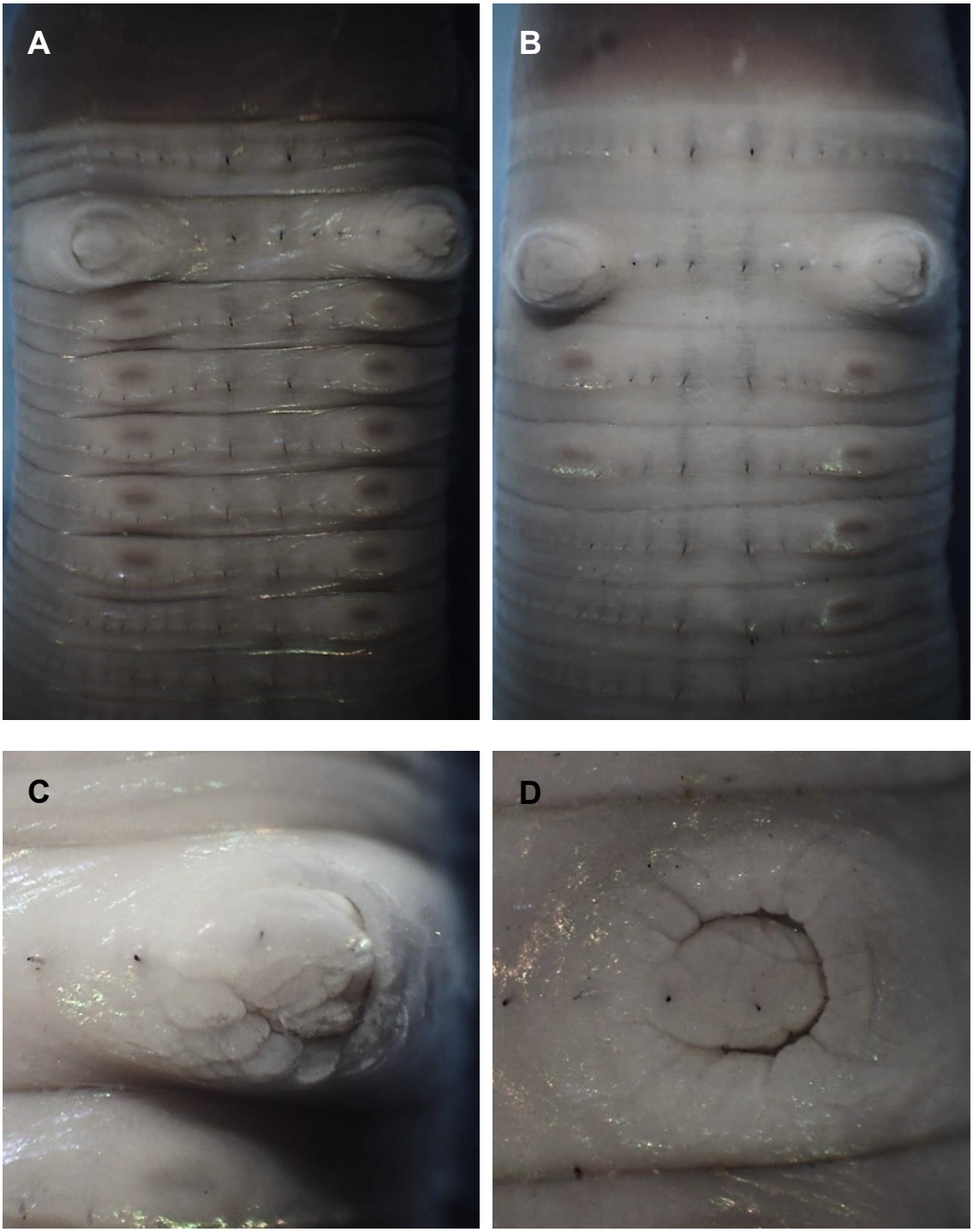


**FIG. 2.18.** *Polypheretima elongata*. (A, B) Male pores and postclitellum genital markings. (C, D) Male pores and copulatory pouches.

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# 3. BELOWGROUND COMPETITION AMONG INVADING DETRITIVORES


## ABSTRACT

The factors regulating soil animal communities are poorly understood. Current theory favors niche complementarity and facilitation over competition as the primary forms of non-trophic interspecific interaction in soil fauna; however, competition frequently has been suggested as an important community-structuring factor in earthworms, ecosystem engineers that influence belowground processes. To date, direct evidence of competition in earthworms is lacking due to the difficulty inherent in identifying a limiting resource for saprophagous animals. In the present study, we offer the first direct evidence of interspecific competition for food in this dominant soil detritivore group by combining field observations with laboratory mesocosm experiments using $^{13}C$ and $^{15}N$ double-enriched leaf litter to track consumption patterns. In our experiments, the Asian invasive species, *Amynthas hilgendorfi*, was a dominant competitor for leaf litter against two European species currently invading the temperate deciduous forests in North America. This competitive advantage may account for recent invasion success of *A. hilgendorfi* in forests with established populations of European species, and we hypothesize that specific phenological differences play an important role in determining the outcome of the belowground competition. In contrast, *Eisenoides lonnbergi*, a common native species in Eastern United States, occupied a unique trophic position with limited interactions with other species, which may contribute to its persistence in habitats dominated by invasive species. Furthermore, our results supported neither the hypothesis

that facilitation occurs between species of different functional groups nor the hypothesis that species in the same group exhibit functional equivalency in C and N translocation in the soil. We propose that species identity is a more powerful approach to understand earthworm invasion and its impacts on belowground processes.

## INTRODUCTION

The importance of the belowground subsystem on ecosystem processes and properties has received increasing recognition (Wardle et al. 2004) particularly with interests concerning invasive species (Belnap et al. 2005), land use and management (de Vries et al. 2013), and different scenarios of temperature, precipitation and $CO_2$ concentration changes (Tylianakis et al. 2008). Belowground biota is the major driver of decomposition (Gessner et al. 2010) and nutrient availability (Bardgett and Wardle 2010). They interact with aboveground components, affect plant productivity (Partsch et al. 2006), diversity and community dynamics (De Deyn et al. 2003), and modulate plant diversity effects on productivity (Eisenhauer et al. 2012) with positive and negative feedbacks between plants and soil organisms (Wardle 2006).

Belowground communities frequently have higher species diversity than the corresponding aboveground systems (Wardle 2006); however, the factors that regulate soil animal communities are poorly understood (De Deyn and Van der Putten 2005; Wardle 2006). At local scales, interspecific interactions within a trophic group, especially competition, in theory, can play an important role in structuring communities that are resource-regulated. However, most groups of soil fauna apparently coexist in high species richness with little sign of competitive exclusion (Wardle 2006). This pattern,

dubbed the "enigma of soil diversity" (Anderson 1975), led to the conclusion that competition is unlikely to be a major structuring force in soil fauna communities. Instead, niche complementarity and facilitation have been proposed as the primary forms of interspecific interaction in soil fauna (Wardle 2006; Hedde et al. 2010).

If exceptions to the "enigma of soil diversity" exist, they might occur in taxa with low species richness at local scales, such as earthworms. Earthworms (Annelida: Clitellata) are the dominant animal group and ecosystem engineers in temperate soil; their activity strongly affects all major soil ecosystem functions, including decomposition, organic matter transformation, biological regulation, and soil engineering (Turbé et al. 2010). Earthworm species richness at local scales is usually limited up to 10-12 species (Decaëns 2010), and is frequently in the range of 4-6 in newly invaded habitats in the temperate region (Hale et al. 2006; Fahey et al. 2013). In this group, competitive exclusion has been frequently suggested to be the major factor structuring its communities (Chauvel et al. 1999; Decaëns 2010). However, direct evidence of competition and its mechanism is still lacking (Uvarov 2009).

Recent studies focusing on natural earthworm assemblages suggest that earthworm communities are highly structured by interspecific interactions, potentially competition (Jiménez et al. 2012). Interspecific competition has been inferred indirectly by differences in maturation rate (Lowe and Butt 2002), growth (Winsome et al. 2006), survival (Abbott 1980), and reproduction (Elvira et al. 1997). However, identification of a limiting resource is necessary to establish presence of competition. Recently, Zhang et al. (2010) proposed a mechanistic explanation in a potential case of competition in which

an Asian invasive species, *Amynthas agrestis*, indirectly impedes the ability of the European species *Lumbricus rubellus* to process leaf litter by reducing soil bacteria abundance, leading to decreased litter consumption and growth. Their explanation, hereafter referred to as the "habitat modification hypothesis", while not supporting competition for food resources, demonstrates that belowground interactions may be more complex than previously thought.

Functional groups are clusters of species playing similar roles in the same ecosystem processes; species in a functional group exhibit functional equivalency and some degree of redundancy to the system (Blondel 2003). Due to this similarity, competition is more likely within the same functional group, and coexistence of species in this case is only possible when temporal and spatial differences exist in their resource exploitation (Ritchie and Olff 1999; Voigt et al. 2007). The most commonly used functional grouping in earthworms is based upon their feeding and burrowing behaviors: "epigeic species" are litter feeders and leaf litter/soil surface dwellers; "endogeic species" are soil feeders and live predominantly in the soil; "anecic species" are litter feeders that live in permanent vertical burrows extending deep into the soil (Bouché 1977). Earthworms in these categories are assumed to affect soil vertical mixing and organic matter translocation differently, and thus these functional groups have been used widely in recent studies focusing on ecosystem functions (Bohlen et a. 2004b; Crumsey et al. 2013).

The ongoing invasion of *Amynthas hilgendorfi*, an Asian species native to Japan, into deciduous forests in the Mid-Atlantic region in the USA provides a unique opportunity to study interspecific interactions with both native and non-native resident species. We

combined laboratory mesocosm experiments using $^{13}C$ and $^{15}N$ double-enriched leaf litter and field observations to test the hypotheses that (1) species from the same functional group, especially epigeic species, compete for food, (2) facilitation occurs between species of different functional groups, (3) competition between the epigeic species is not mediated by soil microbial modification (habitat modification hypothesis), and (4) reduced litter availability due to competition for leaf litter between epigeic species lead to negative, non-additive effects on the translocation of litter-derived fresh C and N into subsurface soil.

## METHODS

### *Field site*

Field sampling was conducted at the Smithsonian Environmental Research Center (SERC), Edgewater, Maryland, USA (38°53'17.0"N, 76°33'14.3"W; www.serc.si.edu). The majority of the upland forests at SERC are composed of successional stands of different ages that had been cleared in the past for agricultural uses and are now part of the Tulip Poplar Association (Brush 1980). The dominant tree species are tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styracifluca*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), box elder maple (*Acer negundo*), American beech (*Fagus grandifolia*), oaks (e.g. *Quercus falcate*, *Q. alba*) and hickories (e.g. *Carya tomentosa*, *C. glabra*) (Higman 1968). Soils at our study location have been classified as Collington sandy loam (fine-loamy mixed, active, mesic Typic Hapludult) (Szlavecz et al. 2011), with an average pH of 5.1, 32% silt, 20% clay, and 5.6% organic matter content (unpublished data).

Earthworms can be found in all forest stands in the Tulip Poplar Association (Szlavecz et al. 2011). Their density, biomass and functional group dominance vary depending on locations and seasons and reach as high as 433 individuals and 155 g (fresh weight) per square meter (Szlavecz and Csuzdi 2007; Ma et al. 2014). Earthworm assemblages at SERC are generally dominated by non-native European Lumbricidae species, such as *Lumbricus rubellus* Hoffmeister, 1843, *L. friendi* Cognetti, 1904, *Octolasion lacteum* (Orley, 1881) and *Aporrectodea caliginosa* (Savigny, 1826). In old forest stands (150+ years post-agriculture) *O. lacteum* is absent, and a native species, *Eisenoides lonnbergi* (Michaelsen, 1894), is common and sometimes dominant. In 2010, an Asian invasive earthworm, *Amynthas hilgendorfi* (Michaelsen, 1892), was first recorded in an old forest called Treefall. Within two years, *A, hilgendorfi* dominated a large portion of this forest stand (unpublished data).

*Mesocosm experiment with isotopically enriched leaf litter*

To test our hypotheses of competition and facilitation among species of different functional groups, a laboratory experiment with different earthworm species and species combinations was conducted using $^{13}C$ and $^{15}N$ double-enriched leaf litter as food. Four species of earthworms were selected based on their origins and functional groups: *Amynthas hilgendorfi,* an epigeic species of Asian origin, *Lumbricus rubellus*, an epigeic species from Europe, *Octolasion lacteum* an endogeic species from Europe, and *Eisenoides lonnbergi*, a native endogeic earthworm. All species (Table 3.1), were collected at SERC, and kept in the dark at 17 $^{o}C$ and 40% RH.

Forest soil from the depth of 0-15 cm was collected at SERC, sieved through a 2 mm

sieve, and kept moist in 19 L (5 gallon) buckets at 10 $^{o}$C. $^{13}$C and $^{15}$N double-enriched tulip poplar (*Liriodendron tulipifera*) leaf litter ($\delta^{13}C = 27.10 \pm 0.18$ ‰, $\delta^{15}N = 890.33 \pm 10.95$ ‰ (mean ± SE); Table 3.1) produced in Bernard et al. (2015) with petioles removed was broken by hand and sieved through a 4 mm sieve. Mesocosms consisted of 2 L white plastic containers with perforated lids (14.8 cm D x 14.9 cm H) to retain moisture (Snyder et al. 2013). 1450.0 g sieved and mixed soil was added into the mesocosms. Gravimetric water content was adjusted to 38% first by adding 85.6 ml of water, then misting 43.5 mL of water after spreading 4.0 g (dry weight) of enriched litter on the surface. The amount of leaf litter added equaled 233 g/m$^2$, similar to average autumn litter fall at SERC (unpublished data). Mesocosms were preconditioned in the dark at 40% RH and 17 $^{o}$C for 16 days prior to the addition of earthworms.

A total of 10 treatments and one control, all with six replicates, were set up: four were single-species treatments with each of the four earthworm species and six were two-species treatments with all combinations of the four species. The control contained both soil and litter but no earthworm. Four *A. hilgendorfi*, six *L. rubellus*, twelve *O. lacteum*, and six *E. lonnbergi* individuals were added into the single-species treatments with the respective species. For the two-species treatments, two *A. hilgendorfi*, three *L. rubellus*, six *O. lacteum*, and three *E. lonnbergi* were added into the respective treatments. The numbers of individuals were chosen for each species to take into account both density in the field and individual biomass differences to mimic potential co-occurrence conditions in the field. The average fresh biomass of individuals were 2.16 ± 0.19 g (mean ± SD) for *A. hilgendorfi*, 0.57 ± 0.06 g for *L. rubellus*, 0.24 ± 0.02 g for *O. lacteum*, and 0.86 ± 0.17 g for *E. lonnbergi*. The mesocosms were incubated in the dark under 40% RH at 17 $^{o}$C

for 21 days. Gravimetric water content was adjusted on days 1, 3, 6, 9, 12, 17, and 21 to 35%. At the end of the experiment, earthworms were removed, counted and weighed. Leaf litter was collected, dried at 60 $^{o}$C and weighed. All soil in the mesocosm was collected and divided into the 0-5 cm layer and the lower layer that roughly equaled 5-10 cm. Earthworm specimens were dissected to remove gut content, freeze-dried, and homogenized. Soil was sieved through a 4-mm sieve and homogenized. Subsamples of soil were stored at -20 $^{o}$C for microbial analysis; another subset was dried at 60 $^{o}$C, and ground. Earthworm and soil samples were analyzed for $^{13}$C and $^{15}$N content as described below.

*Soil earthworm modification experiment*

To test the hypothesis of soil modification by *A. hilgendorfi* influencing the feeding of *L. rubellus*, a separate laboratory mesocosm experiment was set up. Details on mesocosm setup and adjustment of water content were the same as above except that here the leaf litter was not isotopically enriched. The experiment was conducted in two phases in a manner similar to Zhang et al. (2010). In the first phase, 1500 g sieved soil and 2.5 g tulip poplar leaf litter were preconditioned for five days, after which either four *A. hilgendorfi* or six *L. rubellus* were added to each mesocosm in six replicates. The mesocosms were incubated for 16 days, and then taken apart and the worms were removed. The *A. hilgendorfi*-preconditioned soil (A-soil) from the six replicates was combined and mixed. The same procedure was followed for *L. rubellus*-preconditioned soil (L-soil). In the second phase, 12 new mesocosms were established with half of them containing 1500 g of A-soil and L-soil, respectively. 6.0 g of leaf litter and five *L.*

*rubellus* were added to each of the 12 mesocosms. This phase of the experiment lasted 27 days. At the end the remaining leaf litter was collected, dried at 60$^{o}$C and weighed, and the earthworms were counted and weighed.

*$^{13}$C and $^{15}$N natural abundance of earthworms, soil and litter*

We used natural abundance of $^{13}$C and $^{15}$N to assess dietary differences of earthworms in their natural habitat and to infer their "isotopic niches" (Newsome et al. 2007; Jackson et al. 2011). In September 2011 and in August 2013, earthworms, soil and leaf litter were collected at the Treefall forest stand at SERC. The two sampling occasions reflected conditions before and after extensive invasion by *A. hilgendorfi*. Three 1m x 1m quadrats were randomly selected at least 15 m away from each other. After collecting all the leaf litter, earthworms were collected using electroshocking. Three 0-15 cm deep cores were taken from each quadrat using a 5 cm diameter soil corer, and divided into three 5 cm portions. For isotopic analysis earthworms and soil were prepared as described above. Roots and leaf litter were oven dried and ground. Earthworm, soil, roots, and leaf litter samples were analyzed for their $^{13}$C and $^{15}$N (see below).

*Stable isotope analysis*

The C and N elemental and stable isotope composition of freeze dried and powdered earthworm samples from the mesocosm experiment were analyzed at the Purdue Stable Isotope Research Facility (Purdue University, West Lafayette, IN, USA). Specifically, samples were analyzed using a Sercon (Sercon Ltd., Cheshire, UK) GSL combustion

elemental analyzer interfaced to a Sercon 20-22 stable isotope ratio mass spectrometer. The C and N elemental and stable isotope composition of leaf litter and soil samples from the mesocosm experiment were analyzed at the UC Davis Stable Isotope Facility, Davis, CA, USA using either a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) coupled with an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) or a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK). The C and N elemental and stable isotope composition of earthworm, leaf litter, root and soil samples collected from the field were analyzed at the Smithsonian OUSS/MCI Stable Isotope Mass Spectrometry Laboratory, Smithsonian Museum Conservation Institute, Suitland, MD, USA using a Thermo Delta V Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled with a Costech 4010 Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA).

Stable isotope ratios of C and N ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were expressed using the delta ($\delta$) notation: $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample} = [\ R_{sample} / R_{standard} - 1\ ] \times 1000‰$, where $R_{sample}$ is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the samples, and $R_{standard}$ is the isotope ratios in the standard, which is Pee Dee Belemnite (PDB) for C and atmospheric nitrogen for N. The proportion of litter-derived C and N in earthworm tissue was estimated following Balesdent and Mariotti (1996) (See below). Despite homogenization of the soils used in the mesocosms, three out of the sixty earthworm-inoculated soil samples had $\delta^{13}C$ lower than that in the sampled initial soil at the end of the experiment, causing false "negative effects". To accommodate this experimental condition, we excluded soil $\delta^{13}C$ values below that of the initial soil (-27.44‰) from the analysis, leading to exclusion of the

aforementioned three samples.

*Estimating litter-derived C and N in earthworm tissue*

In addition to the $^{13}C$ and $^{15}N$-enriched leaf litter, earthworms may feed on soil-based sources of organic materials that have a natural abundance of the stable isotopes of $^{13}C$ and $^{15}N$ during the 21-day experiment. Soils at the study sites are classified as Collington sandy loam (fine-loamy mixed, active, mesic Typic Hapludult), Monmouth fine sandy loam (fine, mixed, active, mesic Typic Hapludult), or Donlonton fine sandy loam (fine, mixed, active, mesic Typic Hapludults). Our previous work at SERC demonstrates that particle and mineral association of soil organic carbon (SOC) across these sites varies based upon past land use and present earthworm activity (Crow et al. 2009; Ma et al. 2014) and at Treefall stand carbon is primarily associated with silts and clays and intra-microaggregated particles (Ma et al. 2013). Overall, the stable isotope composition of the SOC particles are similar with respect to the $^{13}C$ and $^{15}N$ enriched leaf substrate allowing them to be considered as a single SOC source in an isotope mixing model (unpublished data). Thus the observed changes in earthworm tissue stable isotope signatures can be attributed to feeding on leaf litter or litter-derived C and N sources. The following mixing models can then be used to estimate the proportion of litter-derived C ($f_{C\text{-}ltr}$) and N ($f_{N\text{-}ltr}$) in earthworm tissues:

$$\delta^{13}C_{tissue\text{-}f} = \delta^{13}C_{litter} \times f_{C\text{-}ltr} + \delta^{13}C_{tissue\text{-}i} \times (1 - f_{C\text{-}ltr}) \quad \text{and}$$

$$\delta^{15}N_{tissue\text{-}f} = \delta^{15}N_{litter} \times f_{N\text{-}ltr} + \delta^{15}N_{tissue\text{-}i} \times (1 - f_{N\text{-}ltr}),$$

where $f_{C\text{-}ltr}$ and $f_{N\text{-}ltr}$ are the proportions of litter-derived C and N in earthworm tissues,

$\delta^{13}C_{litter}$ ($\delta^{15}N_{litter}$), $\delta^{13}C_{tissue\text{-}i}$ ($\delta^{15}N_{tissue\text{-}i}$) and $\delta^{13}C_{tissue\text{-}f}$ ($\delta^{15}N_{tissue\text{-}f}$) are the $^{13}C$ ($^{15}N$) abundances in the isotopically-enriched leaf litter, initial earthworm tissues, and earthworm tissues at the end of the experiment, respectively. After rearranging the above two equations:

$f_{C\text{-}ltr} = (\delta^{13}C_{tissue\text{-}f} - \delta^{13}C_{tissue\text{-}i}) / (\delta^{13}C_{ltr} - \delta^{13}C_{tissue\text{-}i})$ and

$f_{N\text{-}ltr} = (\delta^{15}N_{tissue\text{-}f} - \delta^{15}N_{tissue\text{-}i}) / (\delta^{15}N_{ltr} - \delta^{15}N_{tissue\text{-}i})$.

One parameter we intentionally omitted from our calculation is the biological enrichment factors for C and N isotopes that may occur during metabolism. We did this for two reasons. First, the enrichment factors for C and N in earthworms have not been experimentally determined, and our current knowledge from field collected earthworms gives us values that do not reflect the real difference between food and earthworm. Enrichment factors from one trophic level to the next have been generally determined to be about 0-1‰ for $^{13}C$ and 3.4‰ for $^{15}N$ (Post 2002); earthworms, however, usually show $^{13}C$ and $^{15}N$ "enrichment" of 2-4‰ and 0-4‰, respectively, above their potential food sources (Martin et al. 1992; Schmidt et al. 1997; Pollierer et al. 2009). This inconsistency is probably caused by selective ingestion and assimilation of various components in the complex leaf litter and soil organic matter (Martin et al. 1992; Pollierer et al. 2009). Second, our goal is to compare the relative shift of litter-C and N incorporation into the tissue of an earthworm species under different species treatments; omitting the enrichment factor would not affect our estimation of the direction and strength of the relative shift.

*Phospholipid fatty acid analysis*

Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial communities. Samples were prepared and analyzed as described by Buyer and Sasser (2012), using 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) as an internal standard for quantitative analysis. Gas chromatography was conducted on an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmongton, DE, USA) equipped with autosampler, split-splitless injector, and flame ionization detector. The system was controlled with MIS Sherlock (Microbial ID, Inc., Newwark, DE, USA) and Agilent ChemStation software. Fatty acids were identified using the PLFAD1 calibration mix and PLFAD1 peak library (Microbial ID). Random samples were run on a Clarus 500 GC-MS (Perkin-Elmer, Waltham, MA, USA) to confirm fatty acid identifications.

*Statistical analysis*

All statistical tests were conducted in R v3.1.2 (R Core Team 2014). For the mesocosm experiment with isotopically enriched leaf litter, one-way ANOVA followed by Tukey's HSD (honest significant difference) test for multiple comparisons was used to test for species treatment effects on biomass and earthworm tissue $^{13}C$ and $^{15}N$ abundances within each earthworm species. General linear models (GLMs) were used to investigate the effects of earthworm species and species interactions on soil $^{13}C$ and $^{15}N$ abundances using the biomass of each earthworm species average between initial and final weights as independent variables. PLFA were combined into biomarker groups (Buyer and Sasser 2012). The effects of earthworm species and species interactions on Gram-positive, Gram-negative and total bacteria PLFA biomarkers were assessed using GLMs under the same procedure as described above.

For the soil modification experiment, Student's *t*-test was used to assess the effects of soil modification by *A. hilgendorfi* on *L. rubellus* biomass changes and litter consumption.

$\delta^{13}C$ and $\delta^{15}N$ data from field-collected soil, root and earthworm samples were standardized using mean leaf litter (beech and oak) isotopic abundances (Klarner et al. 2014). Differences in the natural abundance of $^{13}C$ and $^{15}N$ of earthworm tissues were used to infer niche differences in a two-dimensional space. The standard ellipse area (SEA) was used to estimate and compare isotopic niche widths as described in Jackson et al. (2011). Compared to the convex hull (Layman et al. 2007), SEA is less sensitive to unequal or small sample sizes (Jackson et al. 2011), allowing us to make robust comparisons between species within a community. In brief, SEA with correction for small sample size ($SEA_c$) was calculated directly from isotope data. A Bayesian SEA ($SEA_B$) with $10^4$ posterior draws was calculated to statistically compare isotopic niche widths and overlaps between species belonging to the same functional group (epigeic or endogeic). The analyses were conducted in SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011) implemented in the package SIAR (Parnell et al. 2008).

## RESULTS

### *Lab mesocosm experiment with $^{13}C$ and $^{15}N$ enriched leaf litter*

In 42 of the 60 mesocosms all earthworms survived. Two mesocosms were excluded due to 50% mortality rate. Biomass of surviving individuals of *O. lacteum* was reduced in the presence of *A. hilgendorfi* ($F_{3,20} = 9.802$, $P = 0.003$) in comparison to all other *O. lacteum* treatments (*O. lacteum* [P = 0.046], *L. rubellus* + *O. lacteum* [P = 0.001], and *O.*

*lacteum* + *E. lonnbergi* [P < 0.001]). Earthworm species treatments had no effect on the biomass of *A. hilgendorfi* ($P = 0.14$), *L. rubellus* ($P = 0.27$) and *E. lonnbergi* ($P = 0.59$).

Earthworm species treatments had significant effects on $\delta^{13}C$ and $\delta^{15}N$ in tissues of *A. hilgendorfi* ($\delta^{13}C$: $F_{3, 20} = 9.13$, $P < 0.001$; $\delta^{15}N$: $F_{3, 20} = 8.93$, $P < 0.001$), *L. rubellus* ($\delta^{13}C$: $F_{3, 16} = 28.08$, $P < 0.001$; $\delta^{15}N$: $F_{3, 16} = 21.38$, $P < 0.001$) and *O. lacteum* ($\delta^{13}C$: $F_{3, 20} = 28.86$, $P < 0.001$; $\delta^{15}N$: $F_{3, 20} = 37.18$, $P < 0.001$), but not *E. lonnbergi* ($\delta^{13}C$: $F_{3, 19} = 1.95$, $P = 0.155$; $\delta^{15}N$: $F_{3, 19} = 0.66$, $P = 0.588$). For all species, the direction and relative changes were consistent between $\delta^{13}C$ and $\delta^{15}N$ values. When *E. lonnbergi* was present, $\delta^{13}C$ and $\delta^{15}N$ values increased significantly in *A. hilgendorfi*, *L. rubellus* and *O. lacteum* compared to those in their respective single-species treatments (Fig. 3.1). $\delta^{13}C$ and $\delta^{15}N$ values decreased in both *L. rubellus* and *O. lacteum* with the presence of *A. hilgendorfi*, but the change was not statistically significant for $\delta^{15}N$ values in *L. rubellus*. *L. rubellus* and *O. lacteum* had no effect on each other. *A. hilgendorfi* $\delta^{13}C$ and $\delta^{15}N$ tissue values increased under the presence of either *L. rubellus* or *O. lacteum*, although the result was only significant under *O. lacteum* (Fig. 3.1). The proportions of litter-derived C and N in earthworm tissues were in the range of 1.6−25.1% and 0.3−7.6%, respectively (Table 3.2). When taking the respective single-species treatment as 100%, *L. rubellus* and *O. lacteum* had 26.6 and 31.0% decrease in litter-derived C and 23.9 and 49.2% decreases in litter-derived N in the presence of *A. hilgendorfi* (Table 3.3).

Both the $\delta^{13}C$ and $\delta^{15}N$ values for the mesocosm soil generally showed consistent patterns, but earthworm species effects were more evident for $^{15}N$ because the isotopic difference between the enriched leaf litter and initial earthworm tissue was much larger

for $^{15}N$ than $^{13}C$. The four earthworm species showed species-specific effects on soil $\delta^{13}C$ and $\delta^{15}N$. The native species, *E. lonnbergi*, by itself had no effect on soil $\delta^{13}C$ and $\delta^{15}N$. *L. rubellus* increased $\delta^{13}C$ and $\delta^{15}N$ in both 0-5 cm ($\delta^{13}C$: $F_{1, 56} = 17.331$, $P < 0.001$; $\delta^{15}N$: $F_{1, 59} = 38.715$, $P < 0.001$) and 5-10 cm ($\delta^{13}C$: $F_{1, 56} = 9.015$, $P = 0.004$; $\delta^{15}N$: $F_{1, 59} = 13.708$, $P < 0.001$). *O. lacteum* and *A. hilgendorfi* also significantly increased soil $\delta^{13}C$ and $\delta^{15}N$, but only in 0-5cm ($\delta^{13}C$: $F_{1, 56} = 23.991$, $P < 0.001$; $\delta^{15}N$: $F_{1, 59} = 54.614$, $P < 0.001$) and 5-10 cm ($\delta^{13}C$: $F_{1, 56} = 40.547$, $P < 0.001$; $\delta^{15}N$: $F_{1, 59} = 136.370$, $P < 0.001$), respectively. (Fig. 3.2, Table 3.4). Earthworm species interactions generally had no effects or positive effects on soil $\delta^{13}C$ and $\delta^{15}N$. However, *A. hilgendorfi* x *L. rubellus* and *A. hilgendorfi* x *O. lacteum* interactions had negative effects on soil $\delta^{15}N$, though this was only significant in the former ($F_{1, 59} = 14.970$, $P < 0.001$ at 0-5 cm, Table 3.4).

*A. hilgendorfi* had significant negative effects on Gram-positive ($F_{1, 57} = 4.780$, $P = 0.033$), Gram-negative ($F_{1, 57} = 14.768$, $P < 0.001$) and total bacteria ($F_{1, 57} = 13.153$, $P < 0.001$) PLFA biomarkers in the 0-5 cm soil layer (Fig. 3.3, Tables 5-7). In 5-10 cm, *A. hilgendorfi* x *L. rubellus* interaction had significant negative effect on Gram-positive ($F_{1, 58} = 5.162$, $P = 0.027$) and total bacteria ($F_{1, 58} = 4.716$, $P = 0.034$) PLFA; *L. rubellus* x *E. lonnbergi* interaction had significant positive effect on Gram-negative PLFA ($F_{1, 58} = 5.138$, $P = 0.027$) (Tables 5-7).

*Soil earthworm modification experiment*

Final biomass of *L. rubellus* individuals in the soil modification experiment were 0.54 ± 0.03 g (mean ± SE) in A-soil and 0.60 ± 0.04 g in L-soil, and leaf litter consumption per individual was 0.57 ± 0.05 g and 0.51± 0.06 g in A-soil and L-soil, respectively.

Neither biomass of *L. rubellus* ($t$ = -1.23, df = 10, $P$ = 0.25) nor litter consumption by *L. rubellus* ($t$ = 0.80, df =10, $P$ = 0.44) between the A-soil and L-soil treatments were significantly different.

*Background ecosystem natural abundance $\delta^{13}C$ and $\delta^{15}N$ values of earthworms*

Five earthworm species, *A. hilgendorfi*, *L. rubellus*, *Ap. caliginosa*, *O. cyaneum* and *E. lonnbergi*, were collected at Treefall stand at SERC. In general, the epigeic species, *L. rubellus* and *A. hilgendorfi*, had the lowest $\delta^{13}C$ and $\delta^{15}N$ values, followed by the endogeic species *Ap. caliginosa* and then by the other two endogeic species, *O. cyaneum* and *E. lonnbergi* (Fig. 3.4). All statistical comparisons were conducted for samples collected within the same year. We did not make any comparisons between the two years due to inherent differences in spatial variability of isotope signatures between the two years, potentially caused by differences in local understory vegetation. The Bayesian approach suggested that between the two epigeic species, the isotopic niche width of *A. hilgendorfi* (SEA = 4.82) was larger than that of *L. rubellus* (SEA = 1.18) ($P$ = 0.005), and 23.4% of the SEA of *L. rubellus* overlapped with that of *A. hilgendorfi* (Fig. 3.4B). Isotopic niche widths of the three endogeic species were not significantly different, and the SEA of *E. lonnbergi* overlapped with that of *O. cyaneum* by only 3.1% (Fig. 3.4A).

**DISCUSSION**

Using isotopically ($^{13}C$ and $^{15}N$) double-enriched leaf litter in combination with natural stable isotope abundance soil in lab mesocosms, we observed shifts in isotope ratios of earthworm tissues in paired-species treatments relative to those in single-species

treatments. These shifts indicated that species altered their feeding behavior in the presence of a potential competitor by consuming more or less leaf litter than they would under intraspecific pressure. Given our knowledge of individual species' feeding preferences, the direction of the shifts in isotope ratios, and a recorded decrease in biomass of one species (*O. lacteum* in the presence of *A. hilgendorfi*), we believe that our experiments demonstrated competition for food resources among invasive earthworms.

Specifically, the results support our first hypothesis that the epigeic species, *Amynthas hilgendorfi* and *Lumbricus rubellus*, compete for leaf litter and the latter shows reduced litter assimilation when *A. hilgendorfi* is present. Our earthworm tissue isotope data did not show any evidence of facilitation between epigeic and endogeic species and thus did not support hypothesis 2. In fact, leaf litter consumption, earthworm tissue stable isotopes, litter-C and -N assimilation and fresh organic matter incorporation into soil all indicate that *O. lacteum* behaves more like an epigeic species that feeds on leaf litter and is active primarily in the 0-5 cm depth. This species experienced reduced leaf litter assimilation and even loss of biomass when *A. hilgendorfi* was present, which was similar to *L. rubellus* in the *L. rubellus* + A. *hilgendorfi* treatment.

Since leaf litter is the only primary source of isotopically enriched C and N, an increase in $\delta^{13}C$ or $\delta^{15}N$ in the soil can be used as an indicator for incorporation of litter-derived C and N into soil organic matter due to earthworm activity. As a consequence of the reduced litter consumption by *L. rubellus* and *O. lacteum* under the competitive pressure by *A. hilgendorfi*, translocation of litter-derived N into 0-5 cm soil by *L. rubellus* and *O. lacteum* is negatively affected [hypothesis 4], though the effect is only statistically

significant in *L. rubellus*. Moreover, although *A. hilgendorfi* decreases soil bacteria abundance, soil modification by *A. hilgendorfi* does not affect the biomass of *L. rubellus* and its leaf litter consumption. This contradicts the prediction of the habitat modification scenario [hypothesis 3], and strengthens our hypothesis 1. Altogether our results suggest that when competing for leaf litter against *L. rubellus* or *O. lacteum*, *A. hilgendorfi* is a superior competitor. While our findings need to be confirmed in the field, to our knowledge, this is the first study documenting direct evidence of competition between earthworms and demonstrating food as a potentially limiting resource.

Litter quality may explain the contrasting results in our study and in the habitat modification scenario reported by Zhang et al. (2010). The oak litter used as food source for earthworms in Zhang et al. (2010) is more recalcitrant than the tulip poplar litter, a highly palatable food source, used in our study. It is well known that slowly decomposing litter types, such as oak, need microbial preconditioning before macro-decomposers accept them as food (Lavelle 1997), which may take months in the field (Zicsi et al. 2011). If litter quality does play a role, the mechanisms through which competition takes place between *A. hilgendorfi* and *L. rubellus* will be context-dependent, and is likely to be determined by litter species and therefore plant community composition.

Natural isotopic abundance from SERC suggests that the isotopic niche width of *A. hilgendorfi* is about four times that of *L. rubellus*. The wide range in natural abundance $\delta^{13}C$ values for *A. hilgendorfi* is clear evidence that the Asian invader feeds on a broad range of $C_3$ plant litter. For an annual species that needs to grow from a cocoon to a sexually mature individual in about four months (Greiner et al. 2012), dietary flexibility

is crucial to its survival, and likely contributes to its worldwide success as an invader (Zhang et al. 2010).

At this early stage of *A. hilgendorfi* invasion at SERC, it is unclear whether the new invader will outcompete *L. rubellus,* another non-native species, most likely established a long time ago. In addition to *A. hilgendorfi* being the superior competitor for food resources, about one-fourth of the isotopic niche of *L. rubellus* overlaps with that of *A. hilgendorfi*. In the locations where the two species co-occur, density of *L. rubellus* is low when *A. hilgendorfi* becomes dominant (pers. obs.). This evidence suggests that *A. hilgendorfi* may outcompete *L. rubellus*. However, the fundamental question is whether there are enough stabilizing niche differences between the two species to overcome fitness advantages potentially held by *A. hilgendorfi* (Chesson 2000; Adler et al. 2007; MacDougall et al. 2009). Species-specific differences in phenology play an important role if the two species do coexist. Phenology can promote stabilizing niche differences by allowing the phenologically offset species to acquire unused resources, and, in some cases, lead to fitness advantages (Godoy and Levine 2014). *A. hilgendorfi* is an annual species that overwinters as cocoons. It does not thrive at low temperature and all adult individuals die by the beginning of winter. An unusual long winter in 2014 appeared to delay the *A. hilgendorfi* life cycle by a month and led to a relatively low summer density (pers. obs.). *L. rubellus* has a life span of several years. It is active at the peak of litter fall (late October-early November), when most *A. hilgendorfi* have died, and it stays active throughout the winter in the Mid-Atlantic region, thus having access to abundant leaf litter resources. These species-specific traits and phenology differences between the two species may reduce the relative fitness advantage of *A. hilgendorfi* and lead to

coexistence.

A central question of invasion biology is what drives the coexistence between the invader and native species (Chesson 2000; MacDougall et al. 2009). This question is especially intriguing for earthworm assemblages in eastern deciduous forests where only a few native species, including *E. lonnbergi*, are able to maintain relatively high densities even when non-native earthworms dominate the community. We propose that niche differences are fundamental in the continuing persistence of *E. lonnbergi* under the invasion of both European and *Amynthas* earthworms. *E. lonnbergi* is often classified as an endogeic species, yet there is negligible overlap in the isotopic niches between *E. lonnbergi* and invasive endogeic species in the field; rather, it appears to occupy a unique trophic position. Moreover, in the mixed species experiments, *E. lonnbergi* was not affected by any of the other earthworms. Our finding supports the idea that native soil decomposers with a distinct trophic position could expand into regions previous unoccupied (Melody and Schmidt 2012).

Our results do not support the species equivalency view within a functional group in key ecological processes, and further corroborate the idea that these functional group classifications are context-dependent (Neilson et al. 2000). C and N translocation into soil is viewed as the fundamental difference between epigeic and endogeic earthworms, yet our four species exhibited four different patterns of soil C and N incorporation. Moreover, the endogeic *O. lacteum* behaved most closely to what is expected of an epigeic earthworm. While previous studies suggest that this species feeds on soil (Zicsi et al. 2011) and utilizes old C resources (Ferlian et al. 2014) or soil microorganisms (Marhan

and Scheu 2005), Ferlian et al. (2014) and Xia et al. (2011) also noted that plant material is important to *O. lacteum*. Here we further demonstrated that *O. lacteum* can consume leaf litter in addition to highly decomposed plant material. During collecting earthworms in the field for our experiments, we observed close association between *O. lacteum* and understory plant roots. Fine roots have been shown to be 2-3‰ more enriched in $\delta^{15}N$ compared to leaf litter (Pollierer et al. 2009), a difference similar to the natural isotopic abundance difference observed between *O. lacteum* and the two epigeic species in our study. Recent studies have demonstrated that rhizosphere C flux in temperate deciduous forests is more important for members of the soil food web, including *O. lacteum*, than previously expected (Pollierer et al. 2007; Gilbert et al. 2014), and that relatively fresh C is a significant part in the nutrition of this species. The absence of roots in my mesocosms, combined with the requirement of fresh organic materials, might have caused *O. lacteum* to consume large amount of leaf litter. Such contrasting results from different studies reveal the differences in the physiological and realized niches of *O. lacteum* and the plasticity of this species under different abiotic and biotic factors, especially food resource availability and interspecific competition. Such plasticity can have important implications for the mass as well as the chemical composition of residual surface litter and litter translocation belowground and may help explain previous observations at SERC forest sites of strong heterogeneity in litter consumption and decay chemistry (Filley et al. 2008).

This study adds to a growing body of research calling for a more cautious approach when using functional groups in studying ecological processes. For communities with relatively low species richness at local scales, species identity can be a better alternative

especially when species-specific responses are present (Fong and Fong 2014) or when species equivalency is disrupted due to natural or anthropogenic disturbance (Voigt et al. 2007), such as biological invasion. We argue that using species identity is a more powerful approach in earthworm invasion studies in temperate North America, where most earthworm communities are composed of only 4-6 species (e.g. Hale et al. 2006; Fahey et al. 2013) from a pool of about 10-12 European lumbricids. As belowground C and N biogeochemistry and modeling are the current focus of earthworm invasion studies in North America, incorporating species-specific information on C and N translocation and soil vertical mixing is critical towards a better understanding of the processes involved.

**TABLE 3.1.** Initial isotopic abundance of leaf litter (enriched), soil and earthworms used in the experiment.

| Sample type | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | Isotopically enriched |
|---|---|---|---|
| Leaf litter | 27.10 | 890.33 | Yes |
| Soil | -27.44 | 1.80 | No |
| Earthworm | | | |
| *Amynthas hilgendorfi* | -26.36 | -0.03 | No |
| *Lumbricus rubellus* | -26.58 | -0.71 | No |
| *Octolasion lacteum* | -27.70 | 2.21 | No |
| *Eisenoides lonnbergi* | -23.70 | 4.34 | No |

**TABLE 3.2.** Proportion of litter-derived C and N (%) in the tissue of *Amynthas hilgendorfi*, *Eisenoides lonnbergi*, *Lumbricus rubellus* and *Octolasion lacteum* under different species treatments.

| | | Proportion of litter-derived C (%) | | Proportion of litter-derived N (%) | |
|---|---|---|---|---|---|
| Species | Treatment* | Mean | (SE) | Mean | (SE) |
| *Amynthas hilgendorfi* | Ah only | 8.8 | (0.4) | 1.2 | (0.1) |
| | Ah + El | 11.6 | (0.5) | 2.0 | (0.1) |
| | Ah + Lr | 10.0 | (0.3) | 1.5 | (0.1) |
| | Ah + Ol | 10.9 | (0.4) | 1.8 | (0.1) |
| *Eisenoides lonnbergi* | El + Ah | 2.0 | (0.4) | 0.4 | (0.1) |
| | El only | 2.7 | (0.4) | 0.5 | (0.1) |
| | El + Lr | 2.1 | (0.3) | 0.4 | (0.0) |
| | El + Ol | 1.6 | (0.2) | 0.3 | (0.0) |
| *Lumbricus rubellus* | Lr + Ah | 11.7 | (1.1) | 3.6 | (0.4) |
| | Lr + El | 22.7 | (0.6) | 7.6 | (0.4) |
| | Lr only | 15.9 | (0.6) | 4.7 | (0.2) |
| | Lr + El | 17.7 | (0.8) | 5.5 | (0.3) |
| *Octolasion lacteum* | Ol + Ah | 12.4 | (0.5) | 1.8 | (0.1) |
| | Ol + El | 25.1 | (1.1) | 5.3 | (0.3) |
| | Ol + Lr | 17.9 | (0.8) | 3.3 | (0.2) |
| | Ol only | 18.0 | (1.2) | 3.6 | (0.3) |

***** Ah: *Amynthas hilgendorfi*; Lr: *Lumbricus rubellus*; Ol: *Octolasion lacteum*; El: *Eisenoides lonnbergi*.

**TABLE 3.3.** Changes of litter-derived C and N in the tissue of *Amynthas hilgendorfi*, *Eisenoides lonnbergi*, *Lumbricus rubellus* and *Octolasion lacteum* compared to the respective single species treatments.

| | | Change of litter-derived C (%)[b] | | Change of litter-derived N (%)[b] | |
|---|---|---|---|---|---|
| Species | Treatment[a] | Mean | (SE) | Mean | (SE) |
| *Amynthas hilgendorfi* | Ah + El | 32.5 | (5.4) | 62.6 | (11.5) |
| | Ah + Lr | 14.0 | (3.3) | 21.3 | (9.7) |
| | Ah + Ol | 24.4 | (4.3) | 47.1 | (6.2) |
| *Eisenoides lonnbergi* | El + Ah | -25.4 | (14.3) | -9.7 | (30.1) |
| | El + Lr | -23.0 | (10.2) | -17.2 | (7.0) |
| | El + Ol | -41.0 | (6.0) | -35.4 | (8.4) |
| *Lumbricus rubellus* | Lr + Ah | -26.6 | (6.8) | -23.9 | (9.5) |
| | Lr + El | 42.7 | (4.0) | 59.8 | (7.8) |
| | Lr + Ol | 11.4 | (4.7) | 16.1 | (6.1) |
| *Octolasion lacteum* | Ol + Ah | -31.0 | (3.0) | -49.2 | (2.8) |
| | Ol + El | 39.5 | (6.4) | 48.4 | (8.4) |
| | Ol + Lr | -0.5 | (4.5) | -9.3 | (5.0) |

[a] Ah: *Amynthas hilgendorfi*; Lr: *Lumbricus rubellus*; Ol: *Octolasion lacteum*; El: *Eisenoides lonnbergi*.

[b] Changes expressed as percentages by taking the abundance in the respective single-species treatment as 100%.

**TABLE 3.4.** Results of general linear models testing the effects of species and their interactions on soil $\delta^{13}C$ and $\delta^{15}N$.

| | $\delta^{13}C$ | | | | $\delta^{15}N$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-5 cm | | 5-10 cm | | 0-5 cm | | 5-10 cm | |
| Factor* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Ah | 0.014 | 0.905 | 40.547 | **<0.001** | 0.032 | 0.860 | 136.370 | **<0.001** |
| Lr | 17.331 | **<0.001** | 9.015 | **0.004** | 38.715 | **<0.001** | 13.708 | **<0.001** |
| Ol | 23.991 | **<0.001** | 1.105 | 0.298 | 54.614 | **<0.001** | 0.331 | 0.567 |
| El | 1.035 | 0.313 | 2.754 | 0.103 | 0.794 | 0.377 | 3.864 | 0.054 |
| Ah x Lr | 2.447 | 0.124 | 0.122 | 0.728 | 14.970 | ↓**<0.001** | 3.094 | 0.084 |
| Ah x Ol | 2.226 | 0.141 | 0.591 | 0.445 | 2.921 | 0.093 | 4.275 | **0.043** |
| Ah x El | 3.950 | 0.052 | 7.120 | **0.010** | 4.160 | **0.046** | 5.324 | **0.025** |
| Lr x Ol | 0.905 | 0.346 | 1.659 | 0.203 | 0.336 | 0.564 | 0.933 | 0.338 |
| Lr x El | 8.274 | **0.006** | 6.445 | **0.014** | 6.507 | **0.013** | 2.878 | 0.095 |
| Ol x El | 0.918 | 0.342 | 0.119 | 0.731 | 0.480 | 0.491 | 0.919 | 0.342 |

Notes: Significant effects ($P<0.05$) are given in bold; ↓, significant negative effect.

* Biomass of *Amynthas hilgendorfi* (Ah), *Lumbricus rubellus* (Lr), *Octolasion lacteum* (Ol), *Eisenoides lonnbergi* (El) and their interactions.

**TABLE 3.5.** Results of general linear models testing the effects of species and their interactions on total bacteria PLFA.

| | Bacteria PLFA (nmole/g) | | | | | |
|---|---|---|---|---|---|---|
| | 0-5 cm | | | 5-10 cm | | |
| Factor* | *F* | df | *P* | *F* | df | *P* |
| Ah | 13.153 | 1, 57 | ↓**<0.001** | 0.005 | 1, 59 | 0.943 |
| Lr | 2.081 | 1, 57 | 0.155 | 1.468 | 1, 59 | 0.231 |
| Ol | 0.543 | 1, 57 | 0.464 | 2.418 | 1, 59 | 0.125 |
| El | 3.850 | 1, 57 | 0.055 | 0.061 | 1, 59 | 0.806 |
| Ah x Lr | 0.009 | 1, 57 | 0.927 | 4.716 | 1, 58 | ↓**0.034** |
| Ah x Ol | 0.985 | 1, 56 | 0.325 | 0.080 | 1, 58 | 0.779 |
| Ah x El | 0.219 | 1, 56 | 0.642 | 0.441 | 1, 58 | 0.509 |
| Lr x Ol | 0.049 | 1, 56 | 0.826 | 1.702 | 1, 58 | 0.197 |
| Lr x El | 0.242 | 1, 56 | 0.624 | 3.580 | 1, 58 | 0.063 |
| Ol x El | 0.022 | 1, 56 | 0.883 | 0.070 | 1, 58 | 0.793 |

Notes: Significant effects (P<0.05) are given in bold; ↓, significant negative effect.

* Biomass of *Amynthas hilgendorfi* (Ah), *Lumbricus rubellus* (Lr), *Octolasion lacteum* (Ol), *Eisenoides lonnbergi* (El) and their interactions.

**TABLE 3.6.** Results of general linear models testing the effects of species and their interactions on Gram-positive bacteria PLFA.

| | PLFA (nmole/g) | | | | | |
|---|---|---|---|---|---|---|
| | 0-5 cm | | | 5-10 cm | | |
| Factor* | *F* | df | *P* | *F* | df | *P* |
| Ah | 4.780 | 1, 57 | ↓**0.033** | 3.215 | 1, 59 | 0.078 |
| Lr | 1.160 | 1, 57 | 0.286 | 0.375 | 1, 59 | 0.543 |
| Ol | 2.034 | 1, 57 | 0.159 | 0.390 | 1, 59 | 0.535 |
| El | 2.841 | 1, 57 | 0.097 | 0.090 | 1, 59 | 0.766 |
| Ah x Lr | 0.599 | 1, 56 | 0.442 | 5.162 | 1, 58 | ↓**0.027** |
| Ah x Ol | 1.891 | 1, 56 | 0.175 | 0.002 | 1, 58 | 0.967 |
| Ah x El | 0.469 | 1, 56 | 0.496 | <0.001 | 1, 58 | 0.981 |
| Lr x Ol | 1.730 | 1, 56 | 0.194 | 0.712 | 1, 58 | 0.402 |
| Lr x El | 0.005 | 1, 56 | 0.946 | 0.754 | 1, 58 | 0.389 |
| Ol x El | 0.305 | 1, 56 | 0.583 | 0.081 | 1, 58 | 0.777 |

Notes: Significant effects (P<0.05) are given in bold; ↓, significant negative effect.

***** Biomass of *Amynthas hilgendorfi* (Ah), *Lumbricus rubellus* (Lr), *Octolasion lacteum* (Ol), *Eisenoides lonnbergi* (El) and their interactions.

**TABLE 3.7.** Results of general linear models testing the effects of species and their interactions on Gram-negative bacteria PLFA.

| | PLFA (nmole/g soil) | | | | | |
|---|---|---|---|---|---|---|
| | 0-5 cm | | | 5-10 cm | | |
| Factor* | *F* | df | *P* | *F* | df | *P* |
| Ah | 14.768 | 1, 57 | ↓**<0.001** | 0.827 | 1, 59 | 0.367 |
| Lr | 1.969 | 1, 57 | 0.166 | 1.986 | 1, 59 | 0.164 |
| Ol | 0.036 | 1, 57 | 0.850 | 3.608 | 1, 59 | 0.062 |
| El | 3.155 | 1, 57 | 0.081 | 0.036 | 1, 59 | 0.851 |
| Ah x Lr | 0.357 | 1, 56 | 0.553 | 3.411 | 1, 58 | 0.070 |
| Ah x Ol | 0.345 | 1, 56 | 0.560 | 0.187 | 1, 58 | 0.667 |
| Ah x El | 0.064 | 1, 56 | 0.801 | 0.959 | 1, 58 | 0.332 |
| Lr x Ol | 0.218 | 1, 56 | 0.643 | 1.996 | 1, 58 | 0.163 |
| Lr x El | 0.437 | 1, 56 | 0.512 | 5.138 | 1, 58 | **0.027** |
| Ol x El | 0.295 | 1, 56 | 0.590 | 0.049 | 1, 58 | 0.826 |

Notes: Significant effects (P<0.05) are given in bold; ↓, significant negative effect.

* Biomass of *Amynthas hilgendorfi* (Ah), *Lumbricus rubellus* (Lr), *Octolasion lacteum* (Ol), *Eisenoides lonnbergi* (El) and their interactions.

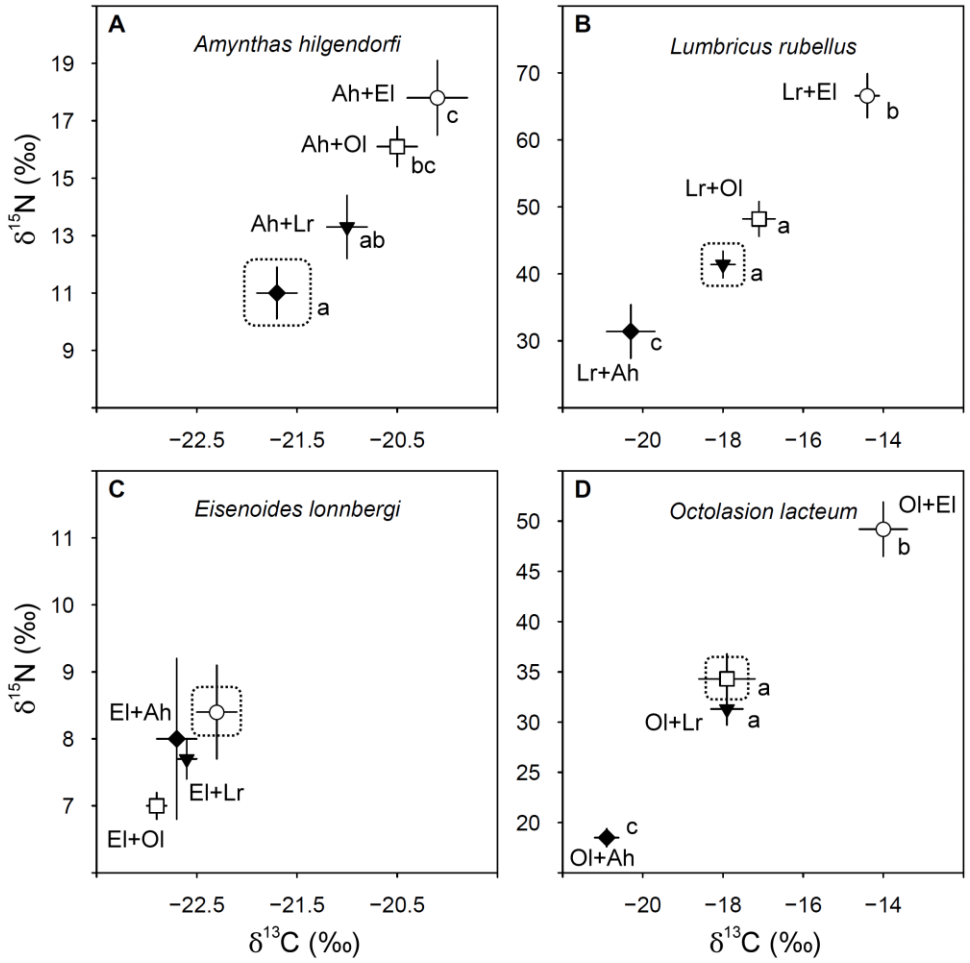


**FIG. 3.1.** $\delta^{13}C$ and $\delta^{15}N$ from (A) *Amynthas hilgendorfi* (Ah), (B) *Lumbricus rubellus* (Lr), (C) *Eisenoides lonnbergi* (El) and (D) *Octolasion lacteum* (Ol) feeding on isotopically enriched leaf litter under different species combination treatments. Values shown are mean and SE. Single-species treatments are circled by dashed squares. For two-species treatments, the treatments are labeled next to the symbols. The same symbols are used for the same "accompanying species": closed diamond, Ah; closed inverted triangle, Lr; open square, Ol; open circle, El. Within each panel, $\delta^{13}C$ with the same letter are not significantly different at $P = 0.05$ (Tukey's HSD test following ANOVA); the same goes for $\delta^{15}N$ except for *L. rubellus* (B), in which the single-species treatment and the Lr + Ah treatments are not significantly different from each other.

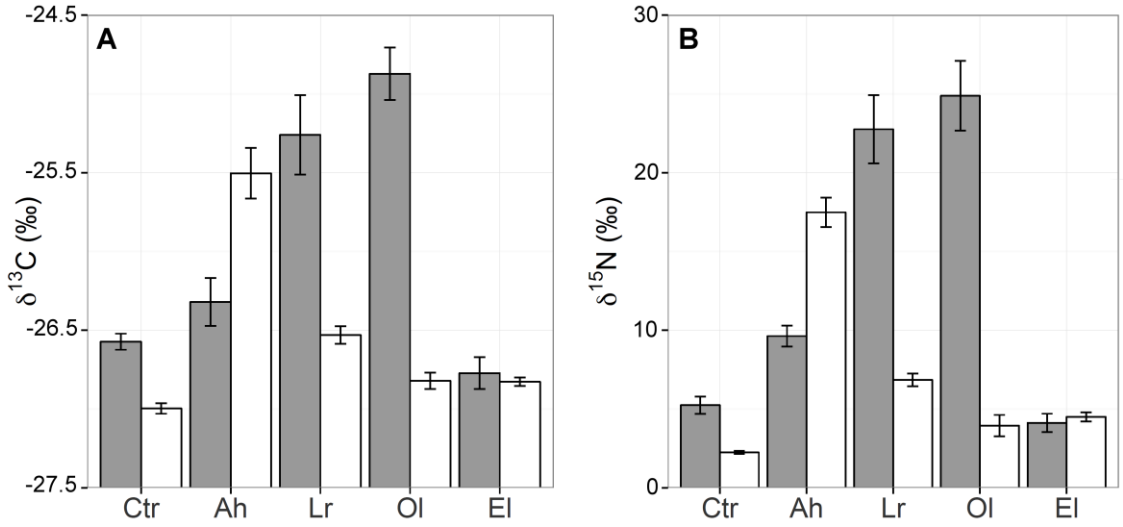


**FIG. 3.2.** $\delta^{13}C$ (A) and $\delta^{15}N$ (B) of bulk soil from 0-5 cm (grey bars) and 5-10 cm (white bars) in the four single-species treatments and the control after the 21-day experiment with isotopically enriched leaf litter. While *L. rubellus* increased $\delta^{13}C$ and $\delta^{15}N$ in both depths, soils with *A. hilgendorfi* and *O. lacteum* were more enriched only in 5-10 cm and 0-5 cm, respectively. Values shown are mean and SE.

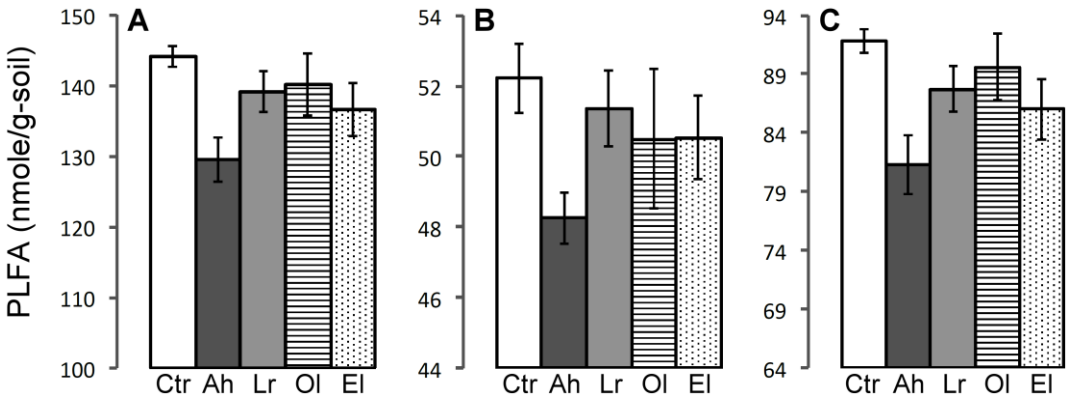


**FIG. 3.3.** Bulk soil (0-5 cm) PLFA biomarker concentrations in the single species treatments containing *A. hilgendorfi*, *L. rubellus*, *O. lacteum* or *E. lonnbergi* and in the control, showing reduced PLFA biomarker concentration in the *A. hilgendorfi* treatment for (A) total bacteria, (B) Gram-positive bacteria and (C) Gram-negative bacteria.

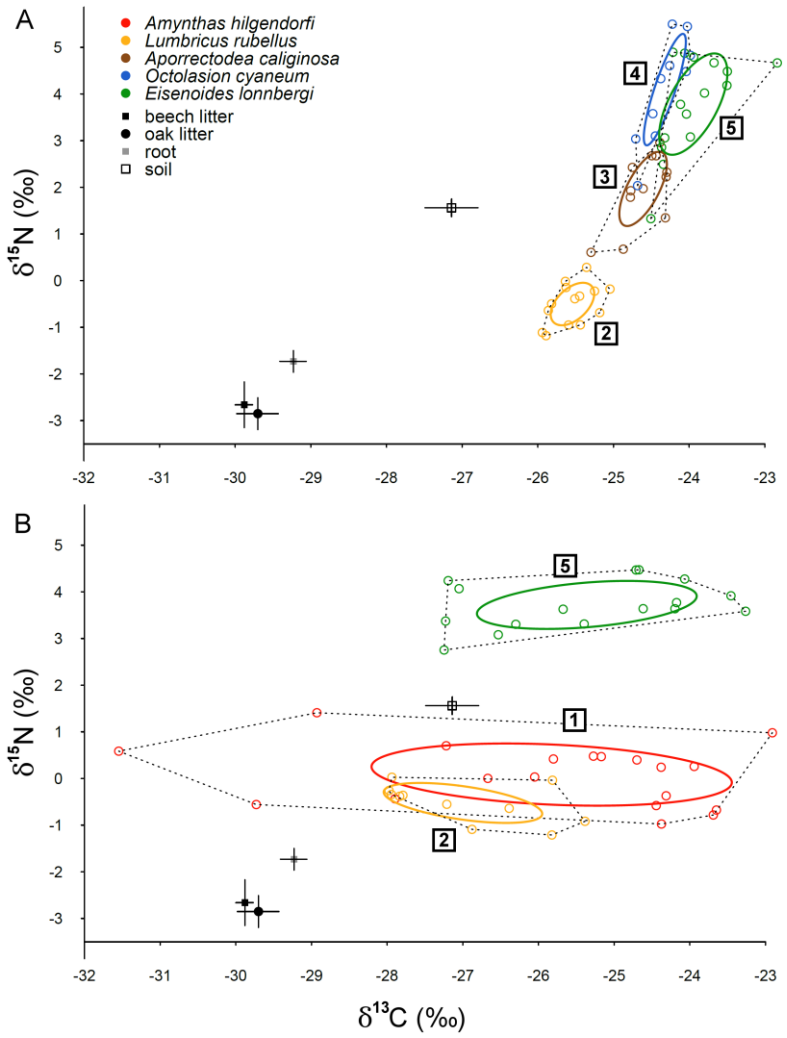


**FIG. 3.4.** Isotopic niche widths of epigeic (*Amynthas hilgendorfi* (1) and *Lumbricus rubellus* (2)) and endogeic earthworm species (*Aporrectodea caliginosa* (3), *Octolasion cyaneum* (4) and *Eisenoides lonnbergi* (5)) inferred from the standard ellipse areas with sample size correction ($SEA_c$) (colored solid lines) based on $\delta^{13}C$ and $\delta^{15}N$. The convex hull of each species (dotted lines) was also shown for comparison. Earthworm specimens were sampled at SERC in (A) 2011 and (B) 2013. The isotope data were standardized using leaf litter (mean of beech and oak). Each colored open circle represents an earthworm individual. Values of leaf litter, roots and soil (mean and standard error) also are presented.

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# 4. SPECIES-SPECIFIC EFFECTS OF EARTHWORMS ON MICROBIAL COMMUNITY, SOIL RESPIRATION AND CARBON TRANSLOCATION


## ABSTRACT

Species in a functional group exhibit functional equivalency and some degree of redundancy to the system. The three earthworm functional groups, anecic, epigeic and endogeic species, are assumed to affect soil vertical mixing and organic matter translocation differently, and thus this classification has been widely used in ecosystem function studies. The objective of this study is to evaluate the fundamental assumption of species equivalency under the context of C biogeochemistry and to further understand the processes through which species-specific responses and interspecific interactions affect soil respiration and how these processed are mediated by soil microbial community. We conducted a lab mesocosm experiment using four earthworm species and $^{13}C$ and $^{15}N$ double-enriched leaf litter, and traced litter-derived C and N into soil and, in the former case, $CO_2$ efflux. Soil respiration was measured regularly throughout the experimental period. Soil microbial community was measured using phospholipid fatty acids (PLFA). Earthworms generally had non-significant effects on soil respiration; however, interspecific interactions between *Octolasion lacteum* (endogeic) and *Lumbricus rubellus* (epigeic) had significant negative effects, presumably through affecting anaerobic microsites in the soil. Litter C-derived soil respiration was reduced by *Amynthas hilgendorfi* (epigeic) and *E. lonnbergi*, but not by *O. lacteum*. Similarly, soil microbial communities were affected by earthworm species identities, but not functional group

affinities, primarily through changing bacteria and fungi abundance. Structural equation models (SEMs) indicated the effects of earthworms on soil C-derived soil respiration were primarily mediated by C and N availability and microbial communities in the soil. In contrast, direct paths between earthworm species and litter C-derived soil respiration implied that microbial activities might be involved in the processes. Path coefficients in the SEMs indicated that the effects of *A. hilgendorfi* on litter C-derived soil respiration, independent of biomass, is stronger than those of the other three species. Altogether, incorporating species-specific responses on soil vertical mixing, C translocation and different components of soil respiration is critical towards a better understanding of earthworm-related C biogeochemistry.

## INTRODUCTION

Since European settlement, temperate deciduous forests in eastern North America have gone through major changes as a result of past land use, climate change and species gains and losses (e.g. Szlavecz and Csuzdi 2007; Nuzzo et al. 2009). Land use changes from primary forests to agriculture provided non-native species the opportunity to invade into habitats with underutilized or unoccupied niches. European earthworm invasion into habitats with low native earthworm abundance or previously devoid of earthworms in this region has raised increasing concerns from researchers and land managers (see below).

Through feeding, burrowing and casting behaviors, invasive European earthworms have been known for negatively affecting seedling survival, reducing the leaf litter layer, and increasing the thickness of organic soil (Hale et al. 2005, 2006, 2008; Dempsey et al. 2011; Dobson and Blossey 2015). These activities also lead to major alterations in soil

properties and C and N biogeochemistry, such as increased bulk density, reduced water retention capacity, changing pH, increased litter decomposition, increased aggregate formation and incorporation of organic matter, decreased soil C, decreased N stock and availability, and increased $CO_2$ and $N_2O$ efflux (Bohlen et al. 2004a; Hale et al. 2005; Bossuyt et al. 2006; Eisenhauer et al. 2007; Szlavecz et al. 2011; Xia et al. 2011; Crumsey et al. 2013; Lubbers et al. 2013; Ma et al. 2013; Dobson and Blossey 2015). In recent years, a group of Asian invasive earthworms, *Amynthas*, has been widely reported invading forests already inhabited by European species, leading to a "second wave of invasion" where the soil ecosystem, already modified by European species, is going through another transition. Two of the invading species, *Amynthas agrestis* and *Amynthas hilgendorfi*, are of special concerns due to their high abundance and biomass (Callaham et al. 2003; Gorres and Melnichuk 2012; Greiner et al. 2012), ability to spread (Bellitürk et al. 2015), and potential to completely displace other earthworm species (Greiner et al. 2012; Chapter 3). While recent studies have shown that the effect of *Amynthas* on soil C may be similar to that of the European species (Snyder et al. 2011, 2013; Greiner et al. 2012), the mechanisms through which different *Amynthas* and European earthworm species affect soil C dynamics is still poorly understood.

Soil respiration is frequently used as a surrogate of ecosystem functions related to C cycles. It is a proxy of integrated biological activities in the soil, primarily root and microbial respirations. Recent studies indicated that the presence of earthworms increases soil respiration by an average of 33% (Lubbers et al. 2013), but the effect is transient and relatively short-term, usually observable only during short-term manipulation experiments (Xia et al. 2011; Crumsey et al. 2013) or at the leading edge of earthworm

invasion front (Eisenhauer et al. 2011). The long-term field study by Fisk et al. (2004) seems to confirm this conclusion, showing no differences in soil respiration between plots with and without earthworms. However, these conclusions do not imply that the underlying processes contributing to soil respiration and their relative importance remain unchanged under earthworm invasion.

The heterotrophic component of soil respiration is primarily the result of decomposition and is driven by microbial communities and activities. Earthworms influence soil microbial communities and activities through complex direct and indirect processes, and the overall effects could be positive, negative or neutral. In general, vertical mixing of leaf litter by epigeic and anecic species tend to increase microbial biomass in the bulk soil as a result of the combination of the release of labile substrates and translocation of readily mineralizable C, while endogeic species tend to decrease microbial biomass in the soil due to their ingestion of recalcitrant organic matter (McLean et al. 2006). Specifically, earthworms may selectively feed on bacteria or bacteria-colonized patches (Jayasinghe and Parkinson 2009; Zirbes et al. 2011), potentially leading to reduced bacteria abundance. The burrowing activities of earthworms can cause disruption of fungal hyphae (Butenschoen et al. 2007), leading to the reduction of fungi. Earthworms can also indirectly affect soil microbes through changing resource availability by consuming leaf litter and soil organic matter (SOM), by casting, and by vertical translocation of C and N in the soil (Eisenhauer et al. 2007). Moreover, the drilosphere, which consists of earthworm burrows, casts and middens, is rich in soluble C and earthworm mucus when fresh and is generally considered hotspots of microbial activities (Aira et al. 2009).

While the effects of earthworms on resource availability and soil microbes have been widely studied (Butenschoen et al. 2007; Jayasinghe and Parkinson 2009; Eisenhauer et al. 2011; Dempsey et al. 2011, 2013; Sackett et al. 2013), less is known about how the different processes involved mediate the effects of earthworms on soil respiration. Moreover, the positive and negative effects of earthworms on soil respiration through different pathways could cancel each other, leading to an overall no effect. For instance, earthworms could positively affect soil respiration by increasing microbial abundance in the drilosphere (Stromberger et al. 2012), but at the same time negatively affect soil respiration by reducing the amount of leaf litter and the microbes living within (Dempsey et al. 2011). Earthworms may also affect the different components of soil respiration through different processes. The incorporation of fresh C and N derived from leaf litter into soil can induce microbial activities and increase mineralization of the litter-derived fresh C. Moreover, the priming effects induced by earthworms may also lead to increased mineralization of order, potentially more recalcitrant C stored in the soil. The latter process has apparent consequences in long-term soil C storage and may be the cause of the observed smaller soil C pools.

Functional groups are clusters of species that play similar roles in specific ecosystem processes (Blondel 2003). The most commonly used functional classification categorized earthworms into three groups based on their feeding and burrowing behaviors: “epigeic species” are litter feeders and leaf litter/soil surface dwellers; “endogeic species” are soil feeders and live predominantly in the soil; “anecic species” are litter feeders that live in permanent vertical burrows extending deep into the soil (Bouché 1977). In general, species in a functional group are assumed to exhibit functional equivalency to the system

(Blondel 2003). The three-category functional group classification of earthworms has been widely used in recent studies focusing on ecosystem functions (Bohlen et al. 2004b; Crumsey et al. 2013), as earthworms of the same group are assumed to affect soil vertical mixing, organic matter translocation, and the subsequent C dynamics similarly. However, results from Chapter 3 demonstrated that common European and Asian species belonging to the same functional group can lead to distinct patterns of organic matter translocation, and the universally recognized endogeic species *Octolasion lacteum* behaved most closely to what is expected of an epigeic earthworm among the species examined, raising concerns regarding the species equivalency assumption in this most commonly adopted functional group classification.

Leaf litter with a unique $^{13}C$ signature, artificially altered through either enrichment or depletion, allows us to trace litter-derived C into soil and $CO_2$ efflux, and to partition soil respiration into litter- and soil-derived components. In this study, we conducted a lab mesocosm experiment using $^{13}C$ and $^{15}N$ double-enriched leaf litter to address the complex processes mediating the effects of earthworms on soil respiration and to evaluate whether earthworm species of the same functional group affect soil respiration similarly through the same mediation processes. We hypothesized that (1) epigeic earthworms affect litter C-derived soil respiration through reducing leaf litter and increasing substrate availability for microbes in the soil, (2) endogeic earthworms reduce soil C-derived soil respiration by reducing microbial biomass in the soil while have no effect on litter C-derived soil respiration, and (3) the interspecific interactions between epigeic and endogeic species have non-additive effects on both litter-C and soil-C derived soil respiration.

## METHODS

*Experimental design*

A laboratory experiment with different earthworm species and species combinations was conducted using $^{13}C$ and $^{15}N$ double-enriched leaf litter as food resources, as described in detail in Chapter 3. Four species of earthworms were selected based on their origins and functional groups: *Amynthas hilgendorfi,* an epigeic species of Asian origin, *Lumbricus rubellus*, an epigeic species from Europe, *Octolasion lacteum* an endogeic species from Europe, and *Eisenoides lonnbergi*, an endogeic earthworm native to North America. All species and forest soil from the depth of 0-15 cm were collected at the Smithsonian Environmental Research Center (SERC), Edgewater, Maryland, USA (38°53'17.0"N, 76°33'14.3"W; www.serc.si.edu).$^{13}C$ and $^{15}N$ double-enriched tulip poplar (*Liriodendron tulipifera*) leaf litter ($\delta^{13}C = 27.1 \pm 0.2$ ‰, $\delta^{15}N = 890.3 \pm 11.0$ ‰ (mean ± SE)) produced in Bernard et al. (2015) with petioles removed was hand-broken and sieved through a 4 mm sieve. Mesocosms consisted of 2 L white plastic containers with perforated lids (14.8 cm D x 14.9 cm H). 1450.0 g sieved and mixed soil was added into the mesocosms followed by 4.0 g of enriched litter on the surface. Gravimetric water content was adjusted to 38%. Mesocosms were preconditioned in the dark at 40% RH and 17 $^{o}C$ for 16 days prior to the addition of earthworms.

A total of 10 treatments and one control, all with six replicates, were set up: four were single-species treatments with each of the four earthworm species (*A. hilgendorfi*, *L. rubellus*, *O. lacteum*, and *E. lonnbergi* treatments, respectively), and six were two-species treatments with all combinations of the four species. The control contained both soil and

litter but no earthworm. Four *A. hilgendorfi*, six *L. rubellus*, twelve *O. lacteum*, and six *E. lonnbergi* individuals were added into the single-species treatments with the respective species. For the two-species treatments, two *A. hilgendorfi*, three *L. rubellus*, six *O. lacteum*, and three *E. lonnbergi* were added into the respective treatments. The numbers of individuals were chosen for each species to take into account both density in the field and individual biomass differences to mimic potential co-occurrence conditions in the field. Three additional mesocosms with no leaf litter and no earthworms ("no-leaf treatment") were constructed for the purpose of isotopic measurements of $CO_2$ gas (see below). The mesocosms were incubated in the dark under 40% RH at 17 $^{o}$C for 21 days. Gravimetric water content was adjusted to 35% on days 3, 6, 9, 12, 17 and 21.

At the end of the experiment, earthworms, remaining leaf litter and soil were collected. Soil samples were divided into the 0-5 cm layer and the lower layer that roughly equaled 5-10 cm, sieved through a 4-mm sieve and homogenized. Subsamples of soil were stored at -20 $^{o}$C for microbial analysis; another subset was dried at 60 $^{o}$C, ground, and analyzed for C and N contents as described below.

*Soil respiration measurement and $CO_2$ gas sampling*

Soil $CO_2$ efflux measurements were taken on days 3, 6, 9, 12, 17 and 21. The mesocosms were put into a customized closed cylinder chamber with a Vaisala GMP343 infrared $CO_2$ sensor (Vaisala, Finland) inserted at the top. $CO_2$ emitted from the soil was accumulated in the headspace of the chamber and the concentration was recorded every second for seven minutes.

$CO_2$ gas was sampled on days 18-20 for stable isotope analysis. Up to 12 mesocosms were randomly selected and sampled at the same time using the same chambers used for $CO_2$ efflux measurements. Vaisala GMP343 infrared $CO_2$ sensors (Vaisala, Finland) were inserted at the top of three of the chambers. The sampling started once $CO_2$ concentration in all three chambers reached 1,000 ppm, and then occurred every 60 min for four hours. At each sampling point, $CO_2$ gas samples were taken using syringes and stored in 12 ml Exetainer vials (LabCo, UK) at room temperature.

*Elemental and stable isotope analyses*

The C and N elemental and stable isotope composition of soil from the mesocosm experiment were analyzed at the UC Davis Stable Isotope Facility, Davis, CA, USA using either a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) coupled with an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) or a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK). $CO_2$ gas from five randomly selected mesocosms of each treatment was analyzed at the same facility using a ThermoScientific PreCon-GasBench system coupled with a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE). Stable isotope ratios of C and N ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were expressed using the delta (δ) notation: $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample} = [ R_{sample} / R_{standard} - 1 ] \times 1000‰$, where $R_{sample}$ is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the samples, and $R_{standard}$ is the isotope ratios in the standard, which is Pee Dee Belemnite (PDB) for C and atmospheric nitrogen for N.

*Phospholipid fatty acid analysis*

Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial communities. Samples were prepared and analyzed as described by Buyer and Sasser (2012), using 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) as an internal standard for quantitative analysis. Gas chromatography was conducted on an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmongton, DE, USA) equipped with autosampler, split-splitless injector, and flame ionization detector. The system was controlled with MIS Sherlock (Microbial ID, Inc., Newwark, DE, USA) and Agilent ChemStation software. Fatty acids were identified using the PLFAD1 calibration mix and PLFAD1 peak library (Microbial ID). Random samples were run on a Clarus 500 GC-MS (Perkin-Elmer, Waltham, MA, USA) to confirm fatty acid identifications.

*Calculation of $CO_2$ flux rates*

$CO_2$ flux rates was calculated using the following equation:

$$F = \frac{\partial C}{\partial t}\left(\frac{PV}{RTS}\right)$$

where $F$ is the gas flux in μmol $m^{-2}$ $s^{-1}$, $\frac{\partial C}{\partial t}$ is the rate of $CO_2$ concentration change in the chamber in ppm $s^{-1}$ (μmol $mol^{-1}$ $s^{-1}$), $P$ is the pressure of headspace gas, $V$ is the volume of the headspace, $R$ is the ideal gas constant (8.314 J $mol^{-1}$ $K^{-1}$), $T$ is temperature in Kelvin, and $S$ is the soil surface area.

$\delta^{13}C$ of $CO_2$ emitted from soil alone and soil plus leaf litter was estimated by applying Keeling plots to the $\delta^{13}C$-$CO_2$ gas data following Pataki et al. (2003). The contribution of litter-derived C ($f_{c\text{-}ltr}$) to $CO_2$ efflux was estimated using a simple isotope mixing model:

$$\delta^{13}C_{gas} = \delta^{13}C_{litter} \times f_{c\text{-}ltr} + \delta^{13}C_{CO2\text{-}soil} \times (1 - f_{c\text{-}ltr})$$

where $f_{c\text{-}ltr}$ is the proportions of litter-derived C in $CO_2$, $\delta^{13}C_{gas}$, $\delta^{13}C_{litter}$ and $\delta^{13}C_{CO2\text{-}soil}$ are the $^{13}C$ abundances in $CO_2$ flux, leaf litter (27.10‰, Chapter 3) and $CO_2$ emitted from soil alone.

The average $CO_2$ flux rate of each treatment was calculated by taking the average of the rates on days 17 and 21, the days before and after the $CO_2$ gas sampling, and was then partitioned into the litter and soil components using estimated $f_{c\text{-}ltr}$.

*Statistical analysis*

All statistical tests were conducted in R v3.1.2 (R Core Team 2014) and, when relevant, using the biomass of each earthworm species as independent variables. Mixed effect models, beta regressions and structural equation modeling (SEM) were conducted using packages lme4 (Bates et al. 2015), betareg (Cribari-Neto and Zeileis 2010) and lavaan (Rosseel 2012), respectively. Data on earthworm survival and biomass, litter consumption, $\delta^{13}C$ and $\delta^{15}N$ of soil, and the absolute abundances of Gram-negative and Gram-positive bacteria PLFA biomarkers have been previously reported in Chapter 3 and were included with the current dataset when necessary.

Cumulative $CO_2$ efflux was analyzed using general linear models (GLMs). The $CO_2$ flux rate at each sampling occasion was analyzed using mixed effect models with sampling occasions as random variables, and likelihood ratio tests were used to access significant differences between nested models. The average $CO_2$ flux rates, $\delta^{13}C$ of $CO_2$ gas emitted from soil, and the partitioned $CO_2$ flux rate derived from only litter or soil were analyzed using GLMs.

GLMs were used to investigate the effects of earthworm species and species interactions on soil C and N contents. PLFA were combined into biomarker groups (Buyer and Sasser 2012) and analyzed using three approaches. First, the effects of earthworms on total PLFA, anaerobe PLFA and bacteria-to-fungi ratio were assessed using GLMs. Second, PLFA data from Gram-negative bacteria, Gram-positive bacteria, Actinomycetes, total bacteria, fungi and protozoa were Hellinger-transformed and analyzed using beta regression, and likelihood ratio tests were used to access significant differences between nested models. Third, microbial community compositions were analyzed using redundancy analyses with the average biomass of earthworm species as constrained variables. Permutation tests with 999 permutations were used to test the significances of the overall models, the constrained axes and the biomass of earthworm species.

In addition to GLMs, we used SEM to investigate how the effects of earthworms on soil respiration were mediated by resource availability and microbial communities. N availability, fresh C availability and litter availability were indicated by soil $\delta^{15}N$, soil $\delta^{13}C$ and remaining litter, respectively. Soil microbial communities were represented

using bacteria and fungi PLFA. The adequacy of models was determined using the likelihood ratio tests and the Akaike information criterion (AIC). Model modification indices were used to improve the models. Further modifications of the model were done by taking into account interspecific interactions and additional potential mediators that were significant in GLMs.

## RESULTS

### *$CO_2$ and soil C & N*

Earthworm species generally had non-significant effects on cumulative $CO_2$ effluxes (Fig. 4.1) and $CO_2$ flux rates. However, interaction between *L. rubellus* and *O. lacteum* reduced both cumulative $CO_2$ efflux ($F_{1, 58} = 5.690$, $P = 0.020$) and $CO_2$ flux rate ($\chi^2 = 6.689$, $P = 0.009$). $\delta^{13}C$ of $CO_2$ gas was significantly reduced by *A. hilgendorfi* ($F_{1, 48} = 181.930$, $P < 0.001$), *L. rubellus* ($F_{1, 48} = 33.049$, $P < 0.001$) and *E. lonnbergi* ($F_{1, 48} = 40.119$, $P < 0.001$), and increased by *L. rubellus* x *E. lonnbergi* interaction ($F_{1, 47} = 6.292$, $P = 0.016$) (Fig. 4.2). On days 17 and 21, the average $CO_2$ flux rate was significantly reduced by *A. hilgendorfi* ($F_{1, 59} = 7.102$, $P = 0.010$), and the litter C-derived $CO_2$ flux rate was significantly reduced by *A. hilgendorfi* ($F_{1, 48} = 29.998$, $P < 0.001$) and by *E. lonnbergi* ($F_{1, 48} = 7.561$, $P = 0.008$). Earthworm species and their interactions had no significant effects on soil C-derived $CO_2$ flux rates. Soil C and N contents were generally not affected by earthworm species. However, *L. rubellus* x *O. lacteum* interaction had a positive effect on C content in 0-5 cm ($F_{1, 57} = 4.461$, $P = 0.039$).

*Microbial communities*

Total PLFA concentration was used as a measure of total microbial biomass. PLFA biomarkers of specific groups were used as measures of biomass of the respective groups. Total PLFA concentration was negatively affected by *A. hilgendorfi* in 0-5 cm ($F_{1, 56}$ = 11.341, $P$ = 0.001). The Hellinger-transformed Gram-negative bacteria PLFA concentration was positively affected by *A. hilgendorfi* in 5-10 cm; the Hellinger-transformed Gram-positive bacteria PLFA concentration was negatively affected by *A. hilgendorfi* but positively affected by *O. lacteum* in 5-10 cm; the Hellinger-transformed Actinomycete PLFA concentration was negatively affected by *A. hilgendorfi* and by *O. lacteum* in 5-10 cm (Table 4.1). The Hellinger-transformed fungi PLFA concentration was positively affected by *A. hilgendorfi* in 5-10 cm and by *O. lacteum* in 0-5 cm (Table 4.1); similarly, the bacteria-to-fungi ratios were reduced by *A. hilgendorfi* at 5-10 cm ($F_{1, 59}$ = 9.268, $P$ = 0.003) and by *O. lacteum* at 0-5 cm ($F_{1, 57}$ = 4.998, $P$ = 0.029). The Hellinger-transformed protozoan PLFA concentration was positively affected by *A. hilgendorfi* in 5-10 cm (Table 4.1). Anaerobe PLFA concentration in 5-10 cm was positively affected by *L. rubellus* x *O. lacteum* interaction ($F_{1, 58}$ = 4.294, $P$ = 0.043). The redundancy analysis (RDA) of microbial community composition indicated that in 0-5 cm the constrained axes (biomass of individual earthworm species) explained 10.9% of the variance, although only axis 1 was significant ($P$=0.003). Among the constrained variables, *A. hilgendorfi* had significant effects ($P$=0.01), while the effects of other earthworm species were not significant (Fig. 4.3A). The biomass of *A. hilgendorfi* is positively associated with Gram-positive bacteria, and negatively correlated with Gram-negative bacteria. RDA of microbial community composition in 5-10 cm showed that the

constrained axes explained 22.3% of the variance; both axis 1 ($P$=0.001) and axis 2 ($P$=0.005) were significant. Among the constrained variables, *A. hilgendorfi* ($P$=0.001) and *O. lacteum* ($P$=0.02) had significant effects (Fig. 4.3B). The biomass of *A. hilgendorfi* is positively associated with Gram-negative bacteria, fungi and protozoa, and negatively associated with Gram-positive bacteria. The biomass of *O. lacteum* is positively associated with Gram-positive bacteria.

*SEM results*

The final model adequately fit the data on soil respiration ($\chi^2_{30}$ = 31.66, P = 0.384; AIC = 1757.5). It explained 55% and 22% of soil respiration derived from litter C and soil C, respectively, 14% of the variance in bacteria PLFA, 12% of fungi PLFA, 52% of soil $\delta^{13}C$, 84% of soil $\delta^{15}N$, and 82% of leaf litter remaining (Fig. 4.4). *A. hilgendorfi*, *L. rubellus* and *E. lonnbergi* had direct negative effects on litter C-derived soil respiration, while only *A. hilgendorfi* negatively influenced soil C-derived soil respiration through a direct path. The four earthworm species had direct negative effects on leaf litter biomass on the soil surface, leading to an increase in $\delta^{13}C$ and $\delta^{15}N$ in the soil. This increased $\delta^{13}C$ led to increased fungi and then bacteria biomass; the former negatively influenced soil C-derived soil respiration, while the latter had positive effects on litter C-derived soil respiration.

**DISCUSSION**

Our results indicated that although earthworms noticeably increased soil respiration during the early phase of the experiment, individual species by themselves had no effects

on either the overall flux rates or cumulative fluxes, supporting the idea that the observed positive effect of earthworms on soil respiration in previous studies may represent transient phenomena (Lubbers et al. 2013). However, even without apparent overall changes in soil respiration, the origins of C in $CO_2$ efflux were distinctively affected by the identities of earthworm species. The proportions of litter C-derived soil respiration were reduced with different degrees by the two epigeic species, *A. hilgendorfi* and *L. rubellus*, and by the endogeic species *E. lonnbergi*, which further led to reduced flux rate in the case of *A. hilgendorfi*. The European endogeic species, *O. lacteum*, had no effects on either the litter C or the soil C-derived soil respiration. Altogether, these results supported the hypothesis that epigeic earthworms affect litter C-derived soil respiration, but provided no evidence for the effects of endogeic species on soil C-derived respiration.

In contrast to hypothesis (3), which expects non-additive effects on litter C and soil C-derived soil respiration between epigeic and endogeic species, most interspecific interactions of earthworms had no effects. However, the negative non-additive effect caused by *L. rubellus* x *O. lacteum* interaction was unexpected. *O. lacteum* has been reported to reduce microbial respiration (Eisenhauer et al. 2007). In my case, it caused reduced soil respiration only when co-occurring with *L. rubellus*, but not with the other epigeic species, *A. hilgendorfi*. The results were consistent with the positive effects of the interaction between *O. lacteum* and *L. rubellus* on soil C content, but contradicted the conclusion by Xia et al. (2011), where interactions between *L. rubellus* and *O. lacteum* increased soil respiration. Two apparent differences between our and Xia et al.'s (2011) experimental designs are the relatively long pre-conditioning period and larger soil mass in our experiment. These conditions, combined with activity of specific earthworm

species, could potentially lead to anaerobic microsites in the soil. Both *L. rubellus* and *O. lacteum* are compacting species, whose activity leads to increased bulk density. We believe that when the two compacting species co-occurred, their activities might have led to increased anaerobic microsites in the soil, causing reduced soil respiration and increased C content. This explanation is supported by the increased anaerobe PLFAs by the *L. rubellus* x *O. lacteum* interaction. This finding indicated that species-specific interactions of earthworms could lead to changes in soil aerobic conditions, impacting aerobic (e.g. $CO_2$) and anaerobic (e.g. $N_2O$) green house gas emissions from the soil.

In Chapter 3, I reported negative effects of *A. hilgendorfi* on biomass of both Gram-positive and Gram-negative bacteria in surface (0-5 cm) soil. However, when relative abundance is taken into account, these effects became marginally or not significant. Instead, a more complex pattern emerged in subsurface (5-10 cm) soil. While *A. hilgendorfi* negatively affected Gram-positive bacteria, including Actinomycetes, the relative abundance of Gram-negative bacteria, fungi and protozoa were all increased by or positively associated with *A. hilgendorfi*. This result is consistent with the finding that some *Amynthas* species may selectively consume Gram-positive bacteria under certain circumstances, leading to reduced Gram-positive bacteria abundance (Zhang et al. 2010). Results in Chapter 3 demonstrated increased fresh C and N translocation from leaf litter into subsurface soil by *A. hilgendorfi*. I believe that this increase in fresh soil organic matter (SOM) is responsible for the increase in the above three microorganism groups. A similar pattern of fresh SOM availability may also explain the increase in fungi by *O. lacteum* in 0-5 cm. However, the increase in fungi biomass and fresh SOM translocation into surface soil by *O. lacteum* contradicted previous finding regarding the same species

(Butenschoen et al. 2007), suggesting that these effects are not only species-specific, but also context-dependent. Litter quality may play a role in the observed differences. While the rye litter in Butenschoen et al. (2007) may not be readily accessible to *O. lacteum*, *O. lacteum* was able to directly consume the high quality tulip poplar litter (see Chapter 3); therefore, more C translocation between litter and soil happened in the latter case as a result of earthworm activity, while in the former case, fungi were the primary facilitator of the vertical movement of litter-derived fresh C.

The final structural equation (SE) model indicated both direct and indirect connections between earthworms and soil respiration. The indirect connections suggested that the effects of both of the epigeic and endogeic earthworms on litter C-derived soil respiration were partially mediated by soil microbial biomass through changing the availability of litter-derived fresh SOM in the soil, while the reduction of leaf litter by earthworms had no direct effect. This result supported our hypothesis (1) that earthworms affect litter C-derived soil respiration through increasing substrate availability for microbes in the soil, and further extended the hypothesis to include both epigeic and endogeic species. However, our results showed no evidence of the reduction of bacteria or fungi in the soil by the two endogeic species, and therefore did not support hypothesis (2) that endogeic earthworms affect soil C-derived soil respiration by reducing microbial biomass.

Direct paths with negative effects between earthworms and litter C-derived soil respiration indicated that the final SE model identified only parts of the important processes involving earthworms. The phenomenon that soil macrofauna negatively affect

litter-C derived soil respiration has been observed in the invasive earthworm *Amynthas corticis* and the millipede *Pseudopolydesmus erasus* native to the US (Snyder et al. 2009), but no hypothesis has been proposed to explain the potential causes. One factor that was not included in the modeling process was microbial activity, which was not represented by the microbial analyses (PLFAs). During the processes of earthworm invasion, microbial activities tend to remain unchanged or even decrease (McLean and Parkinson 1997a, b; Welke and Parkinson 2003; Fisk et al. 2004; Eisenhauer et al. 2011). In my case, I believe that reduced soil microbial activities are responsible for the direct negative effects of *A. hilgendorfi*, *L. rubellus* and *E. lonnbergi* on litter C-derived soil respiration. Presumably a direct path between substrate availability and soil respiration can be a potential indicator of increased biomass-independent microbial activity; the absence of this path in the SE model further supported my hypothesis.

Comparison of the standardized path coefficients of direct paths between earthworms and litter C-derived soil respiration suggested that the effect of *A. hilgendorfi* is larger than those of *L. rubellus* and *E. lonnbergi*. Although *A. hilgendorfi* is larger in body size than *L. rubellus* and *E. lonnbergi*, my analyses have taken this into account and therefore the differences cannot be explained by size alone. *A. hilgendorfi* produces large quantities of granular casts rich in $NH_4^+$ and labile C at the time of excretion. While fresh casts produced by *A. hilgendorfi* have high microbial activities, a week after cast production microbial activities started to decrease (Kawaguchi et al. 2011). These casts become water-stable within 24 hours after formation (Kawaguchi et al. 2011) and increase the prevalence of large soil aggregates in the field (Greiner et al. 2012); moreover, crushing of these casts does not increase C and N mineralization rates (Kawaguchi et al. 2011).

Altogether, these studies indicated that casts of *A. hilgendorfi* form stable aggregates and become low in microbial activities soon after their formation, and therefore may be the cause of the observed stronger effects by *A. hilgendorfi*.

The increase in fungi abundance and decrease in bacteria-to-fungi ratios may explain the unexpected negative direct effect of soil fungi on soil C-derived soil respiration in the SE model. Fungi are generally more efficient at assimilating C compared to bacteria. The increase in fungi abundance and decrease in bacteria-to-fungi ratios can lead to lower C mineralization per unit biomass (Sakamoto and Oba 1994; McLean et al. 2006), and hence the negative effects on soil respiration.

Altogether, my results highlighted that the effects of the four earthworm species on soil respiration are species-specific, and the functional group identities of these species is insufficient in predicting the outcome. While all species affect soil respiration through indirect paths in the final SE model, the effect of *E. lonnbergi* is noticeably smaller than that of the other three species, and species of the same functional group did not shown a consistent pattern through direct paths: the epigeic *A. hilgendorfi* is the only species affecting both litter C and soil C-derived soil respiration; the epigeic species *L. rubellus* and endogeic species *E. lonnbergi*, despite their contrasting litter-feeding and burrowing behaviors, have comparable effects on litter C-derived soil respiration; in contrast, with the absence of a direct path, the effects of *O. lacteum* on both litter C and soil C-derived soil respiration were completely mediated by its consumption of leaf litter and the resulting increase in fresh SOM.

In Chapter 3, I argued that SOM translocation into soil is the fundamental difference between epigeic and endogeic earthworms, yet the patterns of fresh C and N translocation into soil among *A. hilgendorfi*, *L. rubellus*, *O. lacteum* and *E. lonnbergi* do not conform to their functional group identities. For this reason, I proposed that using species identity is a more powerful approach than functional groups in earthworm invasion studies in temperate North America, as most earthworm communities in this region are composed of only 4-6 species from a pool of about 10-12 European lumbricids, making species identification feasible and relatively easy. The result herein further strengthened this argument by showing that in addition to C and N translocation, the four species, which belong to only two functional groups, lead to four distinct patterns of microbial communities and soil respiration, with interspecific interactions causing non-additive effects in both measures. More importantly, changes in these processes and properties have tremendous effects on ecosystem functions; only species identities, but not functional groups, provide enough specific information on vertical soil mixing, organic matter translocation, and microbial communities and activities to address future challenges on uncovering the underlying processes.

**TABLE 4.1.** Results of beta regressions testing the effects of species and their interactions on Hellinger-transformed PLFA biomarkers for bacteria, fungi and protozoa.

| | Gram-negative bacteria | | | | Gram-positive bacteria | | | | Actinomycete | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-5 cm | | 5-10 cm | | 0-5 cm | | 5-10 cm | | 0-5 cm | | 5-10 cm | |
| Factor* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| Ah | 2.887 | ↓0.089 | 16.181 | **<0.001** | 2.687 | 0.101 | 7.402 | ↓**0.007** | 0.282 | 0.596 | 8.142 | ↓**0.004** |
| Lr | 0.009 | 0.926 | 1.014 | 0.314 | 0.226 | 0.634 | 1.080 | 0.299 | 2.259 | 0.133 | 0.284 | 0.594 |
| Ol | 2.121 | 0.145 | 0.585 | 0.445 | 1.612 | 0.204 | 4.383 | **0.036** | 0.550 | 0.458 | 9.040 | ↓**0.003** |
| El | 0.003 | 0.959 | 0.247 | 0.619 | <0.001 | 0.986 | 0.018 | 0.893 | 0.295 | 0.587 | 3.588 | 0.058 |
| Ah x Lr | 4.352 | ↓**0.037** | 0.422 | 0.516 | 1.206 | 0.272 | 0.246 | 0.620 | 2.053 | 0.152 | 0.821 | 0.365 |
| Ah x Ol | 1.251 | 0.263 | 0.051 | 0.822 | 0.515 | 0.473 | 0.637 | 0.425 | 0.800 | 0.370 | 1.372 | 0.241 |
| Ah x El | 0.916 | 0.339 | 3.000 | 0.083 | 0.086 | 0.770 | 0.941 | 0.332 | 1.211 | 0.271 | 0.696 | 0.404 |
| Lr x Ol | 1.493 | 0.222 | 0.383 | 0.536 | 4.517 | ↓**0.034** | 0.599 | 0.439 | 1.810 | 0.179 | 0.095 | 0.758 |
| Lr x El | 1.221 | 0.269 | 1.588 | 0.208 | 0.112 | 0.738 | 3.195 | 0.074 | 2.440 | 0.118 | 1.373 | 0.241 |
| Ol x El | 2.171 | 0.141 | 0.112 | 0.738 | 1.157 | 0.282 | 0.129 | 0.719 | 0.024 | 0.877 | 2.015 | 0.156 |
| | Total bacteria | | | | Fungi | | | | Protozoa | | | |
| | 0-5 cm | | 5-10 cm | | 0-5 cm | | 5-10 cm | | 0-5 cm | | 5-10 cm | |
| Factor* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| Ah | 0.187 | 0.665 | 1.450 | 0.229 | 0.380 | 0.538 | 8.835 | **0.003** | 0.002 | 0.965 | 7.670 | **0.006** |
| Lr | 1.114 | 0.291 | 0.095 | 0.758 | 1.021 | 0.312 | 2.430 | 0.119 | 1.271 | 0.260 | 2.183 | 0.140 |
| Ol | 0.003 | 0.954 | 5.837 | **0.016** | 4.840 | **0.028** | 0.324 | 0.569 | 2.244 | 0.134 | 2.753 | 0.097 |
| El | <0.001 | 0.998 | 0.768 | 0.381 | 2.440 | 0.118 | 3.542 | 0.060 | 0.768 | 0.381 | 3.058 | 0.080 |
| Ah x Lr | 1.152 | 0.283 | 0.016 | 0.899 | 0.620 | 0.431 | 0.214 | 0.643 | 5.577 | ↓**0.018** | 3.560 | 0.059 |
| Ah x Ol | 0.135 | 0.713 | 0.974 | 0.324 | 0.247 | 0.619 | 1.461 | 0.227 | 4.129 | ↓**0.042** | 0.363 | 0.547 |
| Ah x El | 0.726 | 0.394 | 0.512 | 0.474 | 0.404 | 0.525 | 0.181 | 0.671 | 2.876 | 0.090 | 0.051 | 0.822 |
| Lr x Ol | 3.817 | ↓0.051 | 0.154 | 0.695 | 1.440 | 0.230 | 0.881 | 0.348 | 1.110 | 0.292 | 0.533 | 0.465 |
| Lr x El | 1.366 | 0.243 | 1.437 | 0.231 | 0.014 | 0.906 | 0.054 | 0.817 | 1.926 | 0.165 | 0.015 | 0.903 |
| Ol x El | 0.074 | 0.786 | 1.079 | 0.299 | 0.143 | 0.705 | 1.331 | 0.249 | 0.217 | 0.641 | <0.001 | 0.977 |

Notes: Significant effects ($P < 0.05$) are given in bold; ↓, significant or marginally significant ($0.05 < P < 0.1$) negative effect.
***** Biomass of *Amynthas hilgendorfi* (Ah), *Lumbricus rubellus* (Lr), *Octolasion lacteum* (Ol), *Eisenoides lonnbergi* (El) and their interactions.

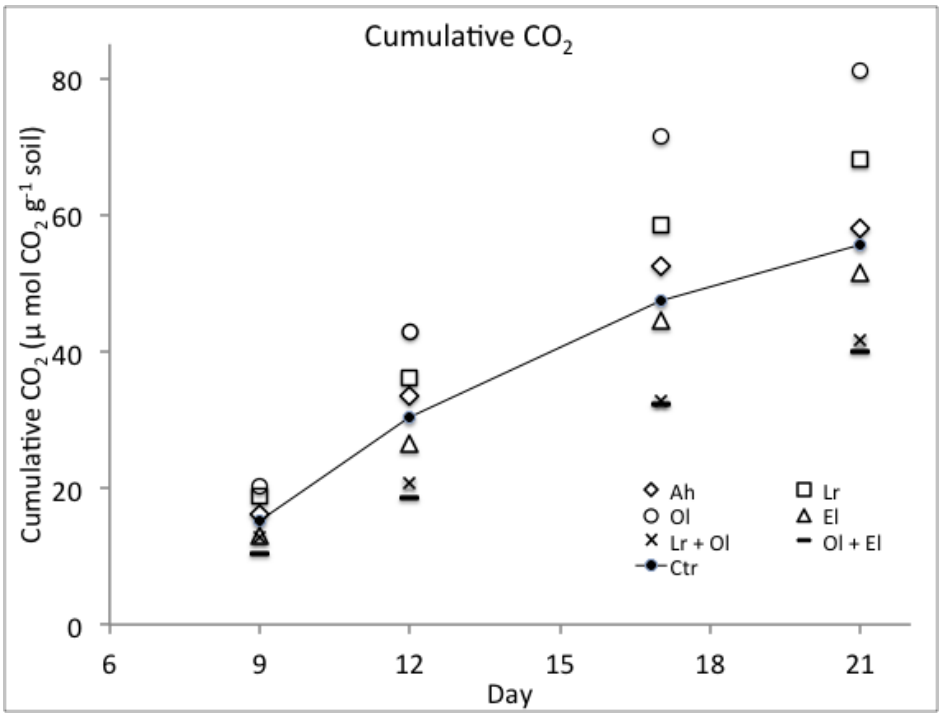


**FIG. 4.1.** Cumulative soil respiration during days 6-21 in Control, single species treatments, and two species treatments. For two species treatments, only those associated with significant ($P$ <0.05) or marginally significant (0.05 < $P$ < 0.1) interactions in the general linear models were shown. Values were means; standard errors were not shown. Ctr: Control; Ah: *Amynthas hilgendorfi*; Lr: *Lumbricus rubellus*; Ol: *Octolasion lacteum*; El: *Eisenoides lonnbergi*.

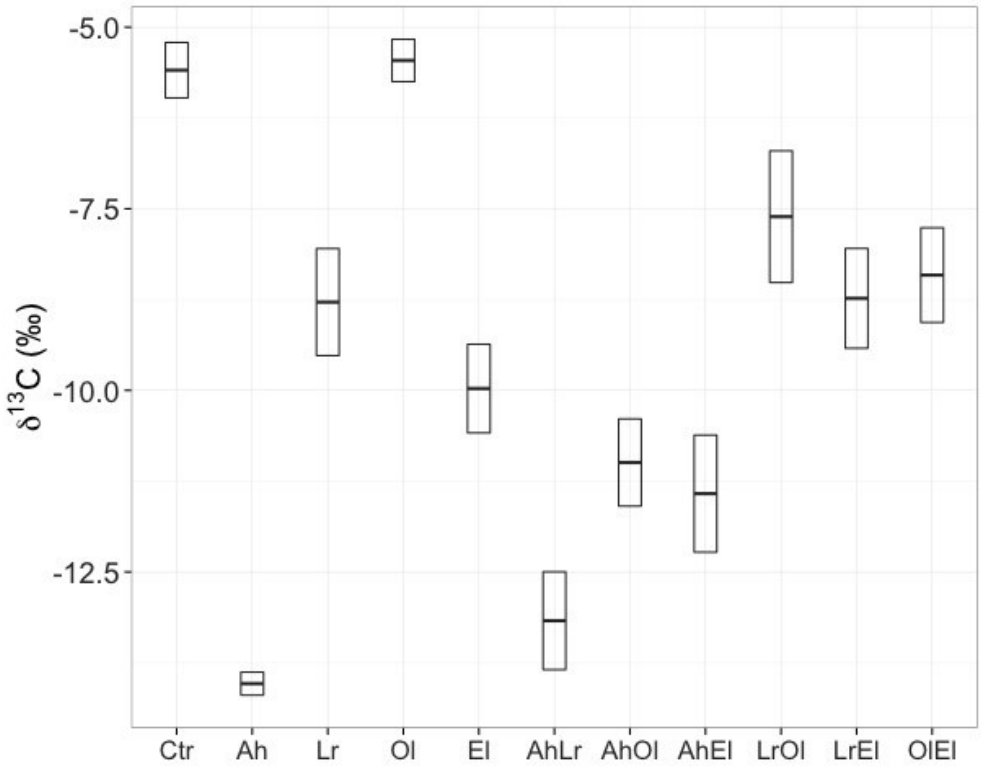


**FIG. 4.2.** $\delta^{13}C$ of soil respiration (soil emitted $CO_2$ gas) from Control, single species treatments, and two species treatments. Each box shows the average and standard error. Ctr: Control; Ah: *Amynthas hilgendorfi*; Lr: *Lumbricus rubellus*; Ol: *Octolasion lacteum*; El: *Eisenoides lonnbergi*.

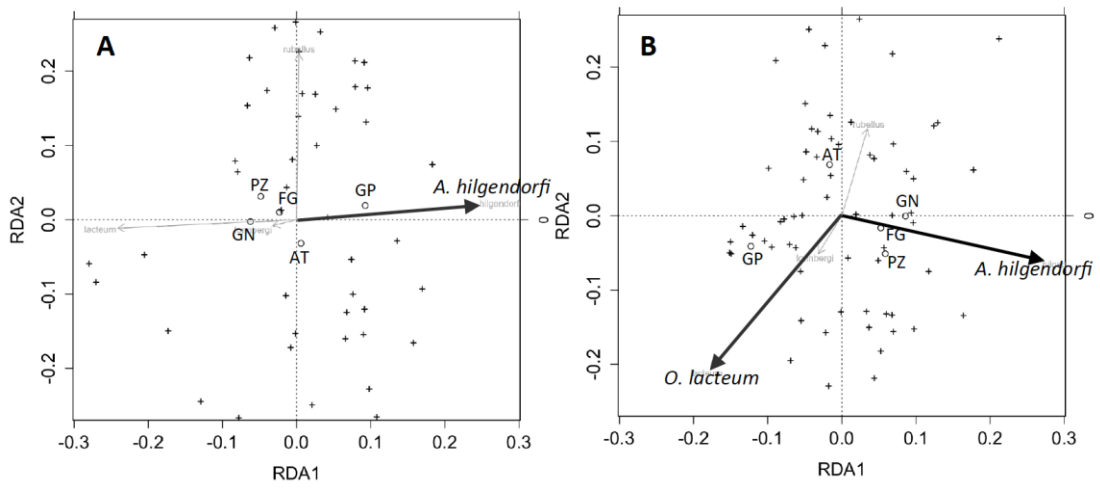


**FIG. 4.3.** Redundancy analysis of soil microbial community structures in 0-5 cm (A) and 5-10 cm (B) soils. Each cross represents a mesocosm. GN: Gram-negative bacteria; GP: Gram-positive bacteria; AT: Actinomycetes; FG: fungi; PZ: protozoa. Significant vectors for earthworm species were highlighted using thick arrows, and vectors for microbes were eliminated for clarity. In 0-5 cm, *Amynthas hilgendorfi* is positively associated with Gram-positive bacteria, and negatively associated with Gram-negative bacteria. In 5-10 cm, *Octolasion lacteum* is positively associated with Gram-positive bacteria; *A. hilgendorfi* is positively associated with Gram-negative bacteria, fungi and protozoa, and negatively associated with Gram-positive bacteria.

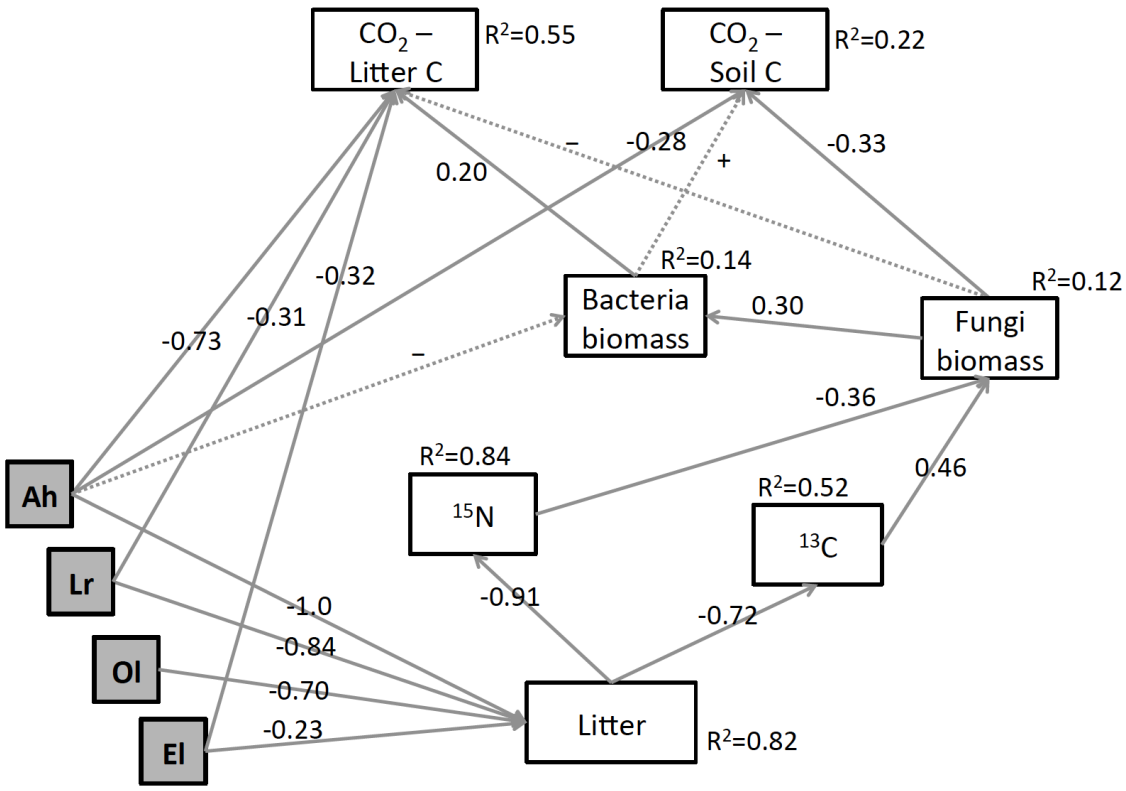


**FIG. 4.4.** Structural equation models showing potential causal effects of earthworm species (exogenous variables; gray square), resource availability and soil microbial abundance on $CO_2$ efflux derived from litter C or soil C (endogenous variables; white rectangles). Ah: *Amynthas hilgendorfi*; Lr: *Lumbricus rubellus*; Ol: *Octolasion lacteum*; El: *Eisenoides lonnbergi*. Numbers on arrows are standardized path coefficients. Arrows with solid lines indicate significant effects ($P < 0.05$); arrows with dashed lines indicate non-significant paths. For the latter, the potential directions of effects were shown using "+" or "-" . Variances explained by the model ($R^2$) were labeled close to each endogenous variable.

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# 5. SUMMARY AND CONCLUSIONS

Invasion of the Asian earthworm genus *Amynthas* into eastern deciduous forests in the United States posses a challenge to both ecologists and land managers regarding understanding their potential impacts and preventing further spreading of the invaders. This challenge is exacerbated by species-specific differences in their life history traits, ecology and effects on ecological processes, such as C translocation and soil respiration. Correct species identification is thus crucial in conducting research and making management decisions. However, identification of *Amynthas* and its related species is relatively difficult compared to the European species, and available taxonomic keys to this group are more than 30 years old and hard to use for ecologists. In this thesis, I presented a new key and diagnosis to species with photos of external characters. As opposed to relying on internal morphology, as the old keys did, the new key is primarily based on external features recognizable under a stereomicroscope without the need for dissection. I also reviewed our current knowledge about the respective species, clarified some common confusions regarding specific names, and highlighted similar species that may be overlooked or lead to misidentification.

One of the species, *Amynthas hilgendorfi*, with its large body size and high biomass, has the potential to transform the soil ecosystems already inhabited by European earthworm species. By using $^{13}C$ and $^{15}N$ double-enriched leaf litter as food resources, I demonstrated that *A. hilgendorfi* is a superior competitor for leaf litter and has the potential to outcompete two common European species, *Lumbricus rubellus* and *Octolasion lacteum*. These interspecific interactions may lead to structural changes in

earthworm communities and further affect ecosystem functions. In contrast to the two European species, the native species *Eisenoides lonnbergi* was not affected by either the Asian or the European species, suggesting that the native species occupies a unique isotopic niche and does not compete with any of the other three species investigated. This potential niche difference can explain why *E. lonnbergi* is one of the few common native species in eastern US and why it does not seem to be threatened by the ongoing earthworm invasion.

In addition to changing earthworm community structures, the microbial components of the soil food web were also significantly affected by *A. hilgendorfi*. The most noticeable changes were in 5-10 cm soil, in which the relative biomass of Gram-negative bacteria, fungi and protozoa was positively affected, but that of Gram-positive bacteria was negative affected. Overall, these changes in bacteria and fungi resulted in a reduced bacteria-to-fungi ratio and potentially shifted the soil food web away from a bacteria pathway-dominated system. These changes were associated with the translocation of litter-derived fresh C and N into subsurface (5-10 cm) soil, and potentially reflected the different responses of different microbial groups under both increased resource availability and increased disturbance caused by the feeding and burrowing behaviors of *A. hilgendorfi*.

While *A. hilgendorfi* did not significantly affect soil respiration, further partitioning of soil respiration revealed a different story. The litter-C component of soil respiration was significantly reduced by *A. hilgendorfi*, *L. rubellus* and *E. lonnbergi*, but the effect was strongest with *A. hilgendorfi*. Further structural equation models suggested that this

strong effect of *A. hilgendorfi* was independent of earthworm biomass, microbial biomass, fresh C and N availability and leaf litter remaining, and was most likely associated with the activities of soil microbes or extracellular enzymes.

My findings called for a more cautious approach when using functional groups in studying ecological processes related to earthworm invasion. Soil organic matter translocation into soil is the fundamental difference between epigeic and endogeic earthworms. However, the four earthworm species examined in this study, which belong to only two functional groups, lead to four distinct patterns of C and N translocation, microbial communities and soil respiration, with interspecific interactions causing non-additive effects in all measures. Moreover, considering C and N translocation, the endogeic European species, *O. lacteum*, behaved most closely to what would be expected from an epigeic species. As changes in the above processes and properties have tremendous effects on ecosystem functions, only species identities, but not functional groups, provide enough specific information on vertical soil mixing, organic matter translocation, and microbial communities and activities to address future challenges on uncovering the underlying processes. With the generally low earthworm species richness at local scales in eastern North America and the available key to *Amynthas* species provided herein, researchers studying earthworm invasion should abandon the simple, three-functional group classification and adopt a species identity-centered approach in studying ecological processes and ecosystem functions.

Alltogether, my research demonstrated the impacts of the Asian invasive earthworm *Amynthas hilgendorfi* on earthworm interspecific interactions, microbial community

structures and soil C and N dynamics. I highlighted the potential impacts of Asian *Amynthas* invasion by showing that *A. hilgendorfi* is a superior competitor against European species and has stronger influences on soil microbial community structures and litter-C derived soil respiration. With this "second wave" of earthworm invasion, the soil ecosystem in the eastern deciduous forests, already modified by European earthworms, is going through another transition.

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Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setala, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**: 1629–1633.

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Willems, J., J. C. Y. Marinissen, and J. Blair. 1996. Effects of earthworms on nitrogen

mineralization. Biology and Fertility of Soils **23**: 57-63.

Winsome, T., L. Epstein, P. F. Hendrix, and W. R. Horwath. 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Applied Soil Ecology **32**: 38–53.

Xia, L., K. Szlavecz, C. M. Swan, and J. L. Burgess. 2011. Inter- and intra-specific interactions of *Lumbricus rubellus* (Hoffmeister, 1843) and *Octolasion lacteum* (Orley, 1881) (Lumbricidae) and the implication for C cycling. Soil Biology & Biochemistry **43**: 1584–1590.

Zhang, W., P. F. Hendrix, B. A. Snyder, M. Molina, J. Li, X. Rao, E. Siemann, and S. Fu. 2010. Dietary flexibility aids Asian earthworm invasion in North American forests. Ecology **91**: 2070-2079.

Zhang, W., P. F. Hendrix, L. E. Dame, R. A. Burke, J. Wu, D. A. Neher, J. Li, Y. Shao, and S. Fu. 2013. Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization. Nature Communications **4**: 2576.

Zicsi, A., K. Szlavecz, and C. Csuzdi. 2011. Leaf litter acceptance and cast deposition by peregrine and endemic European lumbricids (Oligochaeta: Lumbricidae). Pedobiologia **54**: S145–S152.

Zirbes, L., M. Mescher, V. Vrancken, J.-P. Wathelet, F. J. Verheggen, P. Thonart, and E. Haubruge. 2011. Earthworms Use Odor Cues to Locate and Feed on Microorganisms in Soil. Plos One **6**.

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Location of birth: Taipei, Taiwan; date of birth: Jan 15, 1980


## Education

Ph.D. in Ecology, Johns Hopkins University, USA, 2015

Thesis title – The second wave of earthworm invasion: Interspecific interactions, soil microbial communities and carbon cycling.

M.S. in Zoology, National Taiwan University, Taiwan, 2005
B.S. in Zoology, National Taiwan University, Taiwan, 2002

## Research Interests

Soil ecology, C and N cycles, biogeochemistry, ecosystem ecology, stable isotope ecology, community ecology, species coexistence, soil food web, DNA barcoding, molecular phylogeny, earthworm biodiversity, earthworm ecology.

## Positions (2002 – 2015)

2009 – 2015 Graduate Research Assistant, Johns Hopkins University
1.Creating $^{13}C$ & $^{15}N$-labeled leaf litter and applying it in studying competition and biogeochemistry.
2. Aboveground-belowground interactions: invasive earthworms, soil fungi and tree seedlings.
3. The soil-earthworm-litter system controls on the stabilization of soil organic matter.

2011 Research Fellow, Smithsonian Museum of Natural History, Smithsonian Institution, USA
1. Revisiting the taxonomy of the earthworms in the *Aporrectodea caliginosa* species complex.
2. The earthworm genus *Amynthas* and its related species in North America.

2007 – 2009 Research Assistant, National Taiwan University
1. DNA barcoding, systematics and biogeography of the pheretimoid earthworms in Asia.
2. Fauna of Taiwan — Clitellata: Lumbricina and Moniligastrina (earthworms).

2002 – 2005 Graduate Research Assistant, National Taiwan University
1. Systematics and phylogeography of the giant earthworms of the *Metaphire formosae* species group.
2. Earthworm diversity in northern Taiwan.

## Teaching Experience

Teaching Assistant, Johns Hopkins University

270.308 Soil Ecology (2014, 2015).
270.332 Population and Community Ecology (2012, 2014).
270.104 The History of Earth and Its Biota (2010).

Guest Lecturer, Johns Hopkins University

270.332 Population and Community Ecology; '*Ecosystem Ecology'* (2012), '*Decomposition*' (2014).
270.308 Soil Ecology; '*Earthworm Diversity and Ecology*' (2010, 2014).

Guest Lecturer, National Taiwan University, Taiwan

Invertebrate Biology; '*The Systematics and Evolution of Invertebrates*' (2005, 2007 - 2009).

Laboratory Instructor, National Taiwan University, Taiwan

Invertebrate Biology Lab (2003, 2004).

## Grants and Awards

| | |
|---|---|
| 2014 | Robert Balk Fellowship Fund ($3400) |
| 2013 | David Elliott Memorial Fund ($3500) |
| 2012 | Mossom Fieldwork Fund ($3000) |
| 2011 | Smithsonian Institution Fellowship, Smithsonian Institution ($6500) |
| | ESA Travel Award, Ecological Society of America ($300) |
| | IOTM Travel Award, International Oligochaete Taxonomy Society ($650) |
| | Mossom Fieldwork Fund ($2650) |
| 2010 | David Elliott Memorial Fund ($2300) |
| | Eugster Research Fund ($2600) |

## Selected Publications

[25 peer-reviewed papers and one book. See 'Publication List' for the full list.]

Chang, C.-H., Szlavecz, K., Filley, T., Buyer, J., Bernard, M., Pitz, S. (in press) Belowground competition among invading detritivores. Ecology.

Bernard, M., Pitz, S., Chang, C.-H., Szlavecz, K. (in press) Continuous $^{13}C$ and $^{15}N$ labeling of tree litter using a climate-controlled chamber. Communications in Soil Science and Plant Analysis.

Szlavecz, K., Pitz, S.L., Bernard, M.J., Xia, L., O'Neill, J.P., Chang, C.-H., McCormick, M.K., Whigham, D.F. (2013) Manipulating earthworm abundance using electroshocking in deciduous forests. *Pedobiologia* 56, 33-40.

Chang, C.-H., James, S. (2011) A critique of earthworm molecular phylogenetics. *Pedobiologia* 54S, S3-S9.

Chang, C.-H., Lin, S.-M., Chen, J.-H. (2008) Molecular systematics and phylogeography of the gigantic earthworms of the *Metaphire formosae* species group (Clitellata: Megascolecidae). *Molecular Phylogenetics and Evolution* 49, 958-968.

## Selected Presentations

[29 presentations. See 'Presentation List' for the full list.]

Chang, C.-H., Szlavecz, K. (2015) Species-specific effects of detritivores on soil C dynamics. Oral presentation for the "Soil Ecology Society Meeting 2015", June 9-12, 2015, Colorado Springs, CO, USA.

Chang, C.-H., Szlavecz, K., Bernard, M.J., Pitz, S., Filley, T. (2014) Asian earthworm invasion: The stable isotope perspective. The 99th ESA Annual Meeting, August 10-15, 2014, Sacramento, CA, USA.

Szlavecz, K., Chang, C.-H., Bernard, M., McCormick, M., Xia, L., Pitz, S., O'Neill, J., Whigham, D. (2014) The combined effects of land use history and earthworm activity on Mid-Atlantic

forest soil properties. The 10th International Symposium on Earthworm Ecology, June 23-27, 2014, Athens, GA, USA.

Chang, C.-H., Szlavecz, K., Bernard, M.J., Pitz, S., Filley, T. (2013) The second wave of earthworm invasion: a stable isotope perspective on its effect on soil organic matter dynamics. 2013 AGU Fall Meeting, December 9-13, 2013, San Francisco, CA, USA.

Chang, C.-H., Bernard, M.J., Szlavecz, K., Bray, N., McCormick, M.K., Xia, L., Pitz, S., Whigham, D.F., O'Neill, J. (2011) The effects of forest age, earthworm abundance, and leaf litter types on mesofauna and soil properties in Mid-Atlantic deciduous forests. The 96th ESA Annual Meeting, August 7-12, 2011, Austin, TX, USA.

## Professional Activities

*Manuscript Review*

American Midland Naturalist; Biochemical Systematics and Ecology; Endemic Species Research; Journal of Natural History; Molecular Phylogenetics and Evolution; Organisms, Diversity & Evolution; Pedobiologia; PLoS ONE; Raffles Bulletin of Zoology; Taiwan Journal of Biodiversity; Zookeys; Zoological Sciences; Zootaxa.

*Invited talk*

- Asian pheretimoid earthworms in North America. July 23, 2015. Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington D.C., USA.

- Competition among invading earthworms: the stable isotope perspective. June 18, 2015. Smithsonian Environmental Research Center, Edgewater, MD, USA.

- DNA barcoding: a case from earthworms. April 30, 2009. Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland.

*Workshop organized*

- Co-organizer. Life under the City: Assessment of Urban Soils, the 100th ESA Annual Meeting, August 9-14, 2015, Baltimore, MD, USA.

## Professional Development

- Structure Equation Modeling Workshop, August 9-10, Sacramento, CA, USA.
- Soil Acarology Workshop, June 25 - July 13, 2012, Ohio State University, Columbus, Ohio, USA.
- Numerical Analyses of Biological and Environmental Data, May 9-20, 2011, University College London, London, UK.
- Summer Soil Institute, July 14-24, 2010, Colorado State University, Fort Collins, Colorado, USA.

## Professional Affiliations

Ecological Society of America, Soil Ecology Society, American Geophysical Union, International Oligochaete Taxonomy Society.

# Publication List

Peer-Reviewed Papers

*Under review or in preparation*

**Chang, C.-H.**, Szlavecz, K., Buyer, J. The effects of earthworm interspecific interactions on short-term soil C dynamics (To be submitted to Soil Biology and Biochemistry).

**Chang, C.-H.**, Szlavecz, K. A checklist of Asian pheretimoid earthworms in North America with keys to species. (To be submitted to Zootaxa).

*Published or in press*

**Chang, C.-H.**, Szlavecz, K., Filley, T., Buyer, J., Bernard, M., Pitz, S. (in press) Belowground competition among invading detritivores. Ecology.

Bernard, M., Pitz, S., **Chang, C.-H.**, Szlavecz, K. (in press) Continuous $^{13}C$ and $^{15}N$ labeling of tree litter using a climate-controlled chamber. Communications in Soil Science and Plant Analysis.

Shen, H.-P., **Chang, C.-H.**, Chih, W.-J. (2015) Earthworms from Matsu, Taiwan with descriptions of new species of the genera *Amynthas* (Oligochaeta: Megascolecidae) and *Drawida* (Oligochaeta: Moniligastridae). Zootaxa 3973 (3) 425-450.

**Chang, C.-H.**, Chuang, S.-C., Wu, J.-H., Chen, J.-H. (2014) New species of earthworms belonging to the *Metaphire formosae* species group (Clitellata: Megascolecidae) in Taiwan. *Zootaxa* 3774 (4), 324–332.

Shen, H.-P., **Chang, C.-H.**, Chih, W.-J. (2014) Five new earthworm species of the genera *Amynthas* and *Metaphire* (Megascolecidae: Oligochaeta) from Matsu, Taiwan. *Journal of Natural History* 48 (9-10), 495-522.

Szlavecz, K., **Chang, C.-H.**, Burgess, J.L., Csuzdi, C. (2014) Earthworms (Annelida: Clitellata) of Plummers Island, Maryland, U.S.A., with description of a new species. *Proceedings of the Biological Society of Washington* 126 (4) 312-322.

Shen, H.-P., **Chang, C.-H.**, Li, C.-L., Chih, W.-J., Chen, J.H. (2013) Four new earthworm species of the genus *Amynthas* (Oligochaeta: Megascolecidae) from Kinmen, Taiwan. *Zootaxa* 3599 (5), 471–482.

Szlavecz, K., Pitz, S.L., Bernard, M.J., Xia, L., O'Neill, J.P., **Chang, C.-H.**, McCormick, M.K., Whigham, D.F. (2013) Manipulating earthworm abundance using electroshocking in deciduous forests. *Pedobiologia* 56, 33-40.

**Chang, C.-H.**, James, S. (2011) A critique of earthworm molecular phylogenetics. *Pedobiologia* 54S, S3-S9.

Rougerie, R., Decaëns, T., Deharveng, L., Porco, D., James, S.W., **Chang, C.-H.**, Richard, B., Hebert, P.D.N. (2009) DNA barcodes for soil animal taxonomy: transcending the final frontier. *Pesquisa Agropecuaria Brasileira* 44, 789-801.

Lai, Y.-T., **Chang, C.-H.**, Chen, J.-H. (2009) Two new species of *Helobdella* Blanchard 1896 (Hirudinida: Rhynchobdellida: Glossiphoniidae) from Taiwan, with a checklist of hirudofauna of the island. *Zootaxa* 2068, 27-46.

**Chang, C.-H.**, Rougerie, R., Chen, J.-H. (2009) Identifying earthworms through DNA barcodes: pitfalls and promise. *Pedobiologia* 52, 171-180.

**Chang, C.-H.**, Lin, S.-M., Chen, J.-H. (2008) Molecular systematics and phylogeography of the gigantic earthworms of the *Metaphire formosae* species group (Clitellata: Megascolecidae). *Molecular Phylogenetics and Evolution* 49, 958-968.

Shen, H.-P., **Chang, C.-H.**, Chen, J.-H. (2008) A new record of the octochaetid earthworm *Dichogaster affinis* (Michaelsen, 1890) from the centro-western Taiwan. *Endemic Species Research* 10(2), 53-57.

**Chang, C.-H.**, Lin, Y.-H., Chen, I-H., Chuang, C.-S., Chen, J.-H. (2007) Taxonomic re-evaluation of the Taiwanese montane earthworm *Amynthas wulinensis* Tsai, Shen & Tsai, 2001 (Oligochaeta: Megascolecidae): Polytypic species or species complex? *Organisms Diversity and Evolution* 7, 231-240.

Blakemore, R.J., **Chang, C.-H.**, Chuang, S.-C., Ito, M.T., James, S., Chen, J.-H. (2006). Biodiversity of earthworms in Taiwan: a species checklist with the confirmation and new records of the exotic lumbricids *Eisenia fetida* and *Eiseniella tetraedra*. *Taiwania* 51, 226-236.

Lin, Y.-H., **Chang, C.-H.**, Chen, I-H., Chiu, Y.-W., Wu, S.-H., Chen, J.-H. (2006) The survey of the imported aquatic invertebrates via the live aquarium ornamental trade in Taiwan. *Taiwania* 51, 99-107.

**Chang, C.-H.**, Chen, J.-H. (2005) Three new species of octothecate pheretimoid earthworms from Taiwan, with discussion on the biogeography of closely related species. *Journal of Natural History* 39, 1469-1482.

**Chang, C.-H.**, Chen, J.-H. (2005) Taxonomic status and intraspecific phylogeography of two sibling species of *Metaphire* (Oligochaeta: Megascolecidae) in Taiwan. *Pedobiologia* 49, 591-600.

**Chang, C.-H.**, Chuang, S.-C., Chen, Y.-R., Chen, J.-H. (2005) NADH dehydrogenase subunit 1 gene of the earthworm *Amynthas gracilis* (Kinberg, 1867) (Oligochaeta: Megascolecidae), with the discussion on inferring the megascolicid phylogeny using DNA sequences. *Taiwania* 50, 71-75.

**Chang, C.-H.**, Chen, J.-H. (2004) A new species of earthworm belonging to the genus *Metaphire* Sims and Easton 1972 (Oligochaeta: Megascolecidae) from southern Taiwan. *Taiwania* 49, 219-224.

Chen, I.-H., **Chang, C.-H.**, Chuang, S.-C., Lin, Y.-H., Chen, J.-H. (2004) The distribution of the exotic earthworm *Pontoscolex corethrurus* in northern Taiwan and its potential impacts on soil and native earthworm populations. *Chinese Bioscience*, 47 117–126. (in Chinese with English abstract).

Chen, I.-H., **Chang, C.-H.**, Chen, J.-H. (2003) The species composition and distribution of earthworms in Ilan. *Chinese Bioscience* 46, 56–65. (in Chinese with English abstract).

Chuang, S.-C., Wu, J.-C., **Chang, C.-H.**, Chang, C.-H., Yang, K.-W., Lai, W.-S., Wu, Y.-W., Chen, J.-H. (2002) The species composition and distribution of earthworms in northern Taiwan. *Chinese Bioscience* 45, 66–75. (in Chinese with English abstract).

**Chang, C.-H**., Yang, K.-W. Wu, J.-H., Chuang S.-C., Chen, J.-H. (2001) The species composition of earthworms in the main campus of National Taiwan University. *Acta Zoologica Taiwanica* 12, 75-81.

## Books and Book Chapters

**Chang, C.-H.**, Shen, H.-P., Chen, J.-H. (2009). Earthworm Fauna of Taiwan. National Taiwan University Press, Taipei, Taiwan. Pp. 174.

**Chang, C.-H.**, Chen, J.-H. (2008) Earthworm Fauna of Taiwan: *Metaphire formosae* species group. National Taiwan University Press, Taipei, Taiwan. Pp. 60.

**Chang, C.-H.**, Shen, H.-P., Chuang, S.-C., Chen, J.-H. (2008) Earthworm taxonomic studies in Taiwan: a historical review and biodiversity. In: Shao, K.-T., Peng, C.-I, Wu, W.-J. (Eds.), 2008 Taiwan species diversity I. Research and status. Forestry Bureau, Council of Agriculture, Executive Yuan, Taipei, Taiwan, pp 97-108 (in Chinese).

**Chang, C.-H.**, Chen, J.-H. (2008) Phylum Annelida: Class Clitellata: Order Haplotaxoda. In : Shao, K.-T., Peng, C.-I, Wu, W.-J. (Eds.), 2008 Taiwan species diversity II. Species checklist. Forestry Bureau, Council of Agriculture, Executive Yuan, Taipei, Taiwan, pp 658-659 (in Chinese).

Chen, J.-H., **Chang, C.-H.**, Chuang, S.-C., Lai, Y.-T., Lee, C.-L. (2008) Invertebrates in the campus of the National Taiwan University. In: Chen, J.-H., Yuan, H.-W. (Eds.), NTU campus resources, series 1: A guide to the fauna of Taida, National Taiwan University, Taipei, Taiwan. pp. 195-241 (in Chinese).

Theses

**Chang, C.-H.** (2005) Systematics, biogeography and evolution of the earthworms in the *Metaphire formosae* species group inferred from morphology and mitochondrial DNA sequences. M.S. thesis. National Taiwan University. Pp. 101 (in Chinese with English abstract).

**Chang, C.-H.** (2002) Molecular phylogeny of *Amynthas* and *Metaphire* (Clitellata: Megascolecidae) in Taiwan based on 16S rRNA sequences. B.S. thesis. National Taiwan University. Pp. 35 (in Chinese).

# Presentation List

**Chang, C.-H.**, Szlavecz, K., Filley, T., Buyer, J., Bernard, M., Pitz, S. (2015) Belowground competition among invading detritivores. Oral presentation for the "100th ESA Annual Meeting, August 10-14, 2015, Baltimore, MD, USA.

**Chang, C.-H.**, Szlavecz, K. (2015) Species-specific effects of detritivores on soil C dynamics. Oral presentation for the "Soil Ecology Society Meeting 2015", June 9-12, 2015, Colorado Springs, CO, USA.

**Chang, C.-H.**, Szlavecz, K., Bernard, M.J., Pitz, S., Filley, T. (2014) Asian earthworm invasion: The stable isotope perspective. Oral presentation for the "99th ESA Annual Meeting", August 10-15, 2014, Sacramento, CA, USA.

Szlavecz, K., **Chang, C.-H.**, Bernard, M., McCormick, M., Xia, L., Pitz, S., O'Neill, J., Whigham, D. (2014) The combined effects of land use history and earthworm activity on Mid-Atlantic forest soil properties. Oral presentation for the "10th International Symposium on Earthworm Ecology", June 23-27, 2014, Athens, GA, USA.

**Chang, C.-H.**, Szlavecz, K., Bernard, M.J., Pitz, S., Filley, T. (2013) The second wave of earthworm invasion: a stable isotope perspective on its effect on soil organic matter dynamics. Poster presentation for "2013 AGU Fall Meeting", December 9-13, 2013, San Francisco, CA, USA.

Szlavecz, K., Pitz, S., **Chang, C.-H.**, Bernard, M.J. (2013) Creating $^{13}C$- and $^{15}N$-enriched tree leaf litter for decomposition experiments. Poster presentation for "2013 AGU Fall Meeting", December 9-13, 2013, San Francisco, CA, USA.

Shen, H.-P., **Chang, C.-H.** (2013) Reproductive organ degeneration among native Taiwanese *Amynthas* earthworms (Oligochaeta, Megascolecidae) – limitations of species groups of the *Pheretima* complex defined by Sims and Easton (1972). Oral presentation for the "6th International Oligochaete Taxonomy Meeting", Apr. 22-25, 2013, Palmeira de Faro, Portugal.

**Chang, C.-H.**, Bernard, M.J., Szlavecz, K., Bray, N., McCormick, M.K., Xia, L., Pitz, S., Whigham, D.F., O'Neill, J. (2011) The effects of forest age, earthworm abundance, and leaf litter types on mesofauna and soil properties in Mid-Atlantic deciduous forests. Poster presentation for the "96th ESA Annual Meeting", August 7-12, 2011, Austin, TX, USA.

Pitz, S., Szlavecz, K., Xia, L., **Chang, C.-H.**, Bernard, M.J., Carlson, D., Gupchup, J., Houlihan, P. (2011) Soil respiration in an upland tropical rainforest. Poster presentation for the "96th ESA Annual Meeting", August 7-12, 2011, Austin, TX, USA.

Bernard, M.J., Szlavecz, K., Pitz, S., Xia, L., **Chang, C.-H.**, McCormick, M.K., O'Neill, J., Whigham, D.F. (2011) Effectiveness of electroshocking and subsurface barriers when manipulating earthworm populations. Poster presentation for the "96th ESA Annual Meeting", August 7-12, 2011, Austin, TX, USA.

Szlavecz, K., McCormick, M.K., Whigham, D.F., Xia, L., Pitz, S., **Chang, C.-H.**, Bernard, M.J., O'Neill, J. (2011) Combined effects of earthworms and forest age on below- and aboveground processes in the Mid-Atlantic region. Oral presentation for the "96th ESA Annual Meeting", August 7-12, 2011, Austin, TX, USA.

**Chang, C.-H.**, Chih, W.-J., Shen, H.-P., Szlavecz, K., Chen, I.-H., Chuang, S.-C., Chen, J.-H. (2011) Earthworm taxonomy needs DNA barcoding, or *vice versa*? Oral presentation for the "5th International Oligochaete Taxonomy Meeting", April 11-15, 2011, Beatenberg, Switzerland.

James, S., **Chang, C.-H.** (2011) A molecular analysis of diversity within the *Pheretima darnleiensis* species group. Oral presentation for the "5th International Oligochaete Taxonomy Meeting", April 11-15, 2011, Beatenberg, Switzerland.

**Chang, C.-H.**, James, S. (2010) 10 years of earthworm molecular phylogenetics: lessons learned and perspectives. Oral presentation for the "9th International Symposium on Earthworm Ecology", September 5-10, 2010, Xalapa, Veracruz, Mexico.

**Chang, C.-H.**, Szlavecz, K., Pitz, S., Csuzdi, C., McCormick, M., Filley, T. (2010) Invasion of Asian *Amynthas* species and increase of earthworm abundance in Mid-Atlantic deciduous forests of the USA during 1999-2010. Poster presentation for the "9th International Symposium on Earthworm Ecology", September 5-10, 2010, Xalapa, Veracruz, Mexico.

**Chang, C.-H.**, James, S., Chen, J.-H., Shen, H.-P., Szlavecz, K., Chih, W.-J., Liao, J.-M. (2010) Regional earthworm DNA barcode database, BOLD system and their applications: a case study from Taiwan. Poster presentation for the "9th International Symposium on Earthworm Ecology", September 5-10, 2010, Xalapa, Veracruz, Mexico.

**Chang, C.-H.**, Szlavecz, K., Pitz, S., Csuzdi, C., McCormick, M., Filley, T. (2010) Recent increase in earthworm abundance in Mid-Atlantic deciduous forests. Poster presentation for the "95th ESA Annual Meeting", August 1-5, 2010, Pittsburgh, PA, USA.

**Chang, C.-H.** (2009) DNA barcoding: a case from earthworms. Invited talk, April 30, 2009, Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland.

**Chang, C.-H.**, James, S., Shen, H.-P., Chen, J.-H. (2009) The auxiliary of traditional taxonomy: using DNA barcoding in earthworm taxonomy. Oral presentation for "the 4th International Oligochaete Taxonomy Meeting" April 19-26, 2009, Dicle University, Diyarbakir, Turkey.

James, S., **Chang, C.-H.**, Shen, H.-P., Chen, J.-H. (2009) Preliminary molecular systematics of the *Pheretima* complex. Oral presentation for "the 4th International Oligochaete Taxonomy Meeting" April 19-26, 2009, Dicle University, Diyarbakir, Turkey.

**Chang, C.-H.**, Shen, H.-P., Chuang, S.-C., Chen, J.-H. (2008) Earthworm taxonomic studies in Taiwan: a historical review and biodiversity. Oral presentation for "the International Workshop on Research and Status of Taiwan Species Diversity" August 15-16, 2008, Natural Museum of Natural Science, Taichung, Taiwan. (in Chinese)

Rougerie, R., Decaëns, T., Deharveng, L., **Chang, C.-H.**, James, S., Porco, D. Hebert, P.D.N. (2008) DNA barcodes for soil animal taxonomy: transcending the final frontier. Oral presentation for "the 15th International Colloquium on Soil Zoology" August 25-29, 2008, Positivo University, Curitiba, Brazil.

Rougerie, R, Porco, D., James, S., Deharveng, L, **Chang, C.-H.**, Decaëns, T., Bedos, A., Hebert, P.D.N. (2008) DNA barcoding of earthworms and collembolans: opening the 'black box' of soil biodiversity. Poster presentation for "the 2nd Canadian Barcode of Life Network 2008 Scientific Symposium" April 28-29, 2008, Royal Ontario Museum, Toronto, Canada.

Chen, J.-H., **Chang, C.-H.** (2007) Earthworm species identification in gut content analyses of predators. Oral presentation for "the Symposium on Wildlife Conservation and Research" June 28-29, 2007, Biodiversity Research Center, National Taiwan University, Taipei, Taiwan. (in Chinese)

**Chang, C.-H.**, James, S.W., Hong, Y., Shen, H.-P., Chen, J.-H. (2007) Barcoding of earthworms: a case study of the *Pheretima* complex (Clitellata: Megascolecidae). Poster presentation for "the 2nd International Barcode of Life Conference" September 18-20, 2007, Academica Sinica, Taipei, Taiwan.

**Chang, C.-H.**, Chen, J.-H. (2007) Molecular systematics and phylogeography of the gigantic earthworms in the *Metaphire formosae* species group (Oligochaeta: Megascolecidae). Oral and poster presentations for "the 3rd International Oligochaete Taxonomy Meeting" April 2-6, 2007, Pendeli Hotel, Platres, Cyprus.

**Chang, C.-H.**, Chen, J.-H. (2004) Islands on an Island: diversity of gigantic earthworms in Taiwan. Poster presentation for "the Internal Symposium on Impacts of Soil Biodiversity on

Biogeochemical Processes in Ecosystems & Workshop on Molecular Methods in Soil Biological and Biochemical Diversity in Terrestrial Ecosystems", Apr. 18-24, 2004, Taiwan Forestry Research Institute, Taipei, Taiwan.

**Chang, C.-H.**, Chen, J.-H. (2003) Taxonomy, biogeography and phylogeny of the earthworms in the *Metaphire paiwanna* species group. Poster presentation for "the 2003 Symposium on Animal Behavior and Ecology", Feb. 24-28, 2003, Academica Sinica, Taipei, Taiwan (in Chinese).

**Chang, C.-H.**, Chen, J.-H. (2001) Species composition and distribution of earthworms in the main campus of the National Taiwan University. Oral presentation for "the 2001 Symposium on Animal Behavior and Ecology", Feb. 12-13, 2001, Taipei Zoo, Taipei, Taiwan (in Chinese).